ASSESSMENT OF MALTING BARLEY VARIETIES FOR RESISTANCE TO IMPORTANT BARLEY DISEASES IN NEW YORK

A Thesis

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ABSTRACT

In 2013, the State of New York created the Farm Brewery License that requires brewers who benefit from the tax and marketing incentives of the license to use state-grown ingredients, especially hops and barley, in their beer. Malting barley has not been grown in New York for about one hundred years, and, until recently, little was known about which barley pathogens would be problematic and what tools would best work to control them in New York. Genetic resistance is one of the most economical and effective ways to manage plant diseases, so cultivars of winter and spring barley were evaluated for disease resistance to natural populations of the pathogens causing scald (*Rhynchosporium commune*), spot blotch (*Bipolaris sorokiniana*), leaf rust (*Puccinia hordei*), powdery mildew (*Blumeria graminis* f.sp. *hordei*), and Fusarium head blight (*Fusarium* spp.). Data were collected from multiple locations over the course of three field seasons to determine the level of resistance each cultivar exhibited for each disease. In this process, spot blotch was identified as a potentially serious threat to spring barley. To prepare for greenhouse evaluations of spot blotch, an aggressive isolate of *B. sorokiniana* collected in New York was identified, inoculum production techniques were tested, and twelve cultivars were evaluated at seedling and adult growth stages to select susceptible and resistant check varieties. These techniques were implemented to screen a spring two-row barley population composed of diverse elite breeding lines for seedling response to spot blotch to identify quantitative trait loci (QTL) using genome-wide association (GWA) mapping. Two QTL were identified on chromosomes 2H and 7H. The same population was planted in the field, and the lines were evaluated as adults for response to spot blotch, powdery mildew, leaf rust, and Fusarium head blight, using only natural inoculum. One QTL for powdery mildew resistance and two QTL for leaf rust resistance were identified.

BIOGRAPHICAL SKETCH

Alyssa Blachez grew up in Storrs, CT and obtained her high school diploma from Edwin O. Smith High School in 2010. She continued her education at Cornell University, where she obtained her Bachelor of Science degree in Biological Sciences with a concentration in Ecology and Evolutionary Biology in January 2014. In the following spring and summer, she worked as a research assistant in Dr. Rebecca J. Nelson's plant pathology and plant breeding lab at Cornell. In August 2014, she joined Dr. Gary C. Bergstrom's lab in the Plant Pathology and Plant-Microbe Biology section of the School of Integrative Plant Science at Cornell University to complete her master's degree.

Dedicated to my grandfather John C. Cowles who instilled a love of plants and hard work in his children and grandchildren. His spirit lives on in the gardens he left behind.

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CHAPTER 1

A REVIEW OF THE MALTING BARLEY INDUSTRY AND COMMON BARLEY DISEASES IN NEW YORK

The research for this master's thesis was performed in support of the New York State craft brewing industry. In 2012, Governor Andrew Cuomo signed the Farm Brewery Act into law (Office of the Governor Andrew M. Cuomo 2012). The license created by this law provides economic incentives for craft brewers to use New York State-grown products, especially barley and hops, in their beer. Specifically, the Farm Brewery License costs less than the generic brewery license and permits the holder to sell other New York State labeled alcoholic beverages, open restaurants, and sell other related products at their brewery (ABC 4§51-a). In exchange, they must use a certain percentage of hops and other dry ingredients grown in-state in their beer. The percentage is scheduled to increase progressively until 2024 when Farm Brewery License holders will have to use at least 90% of hops and 90% of all other dry ingredients grown in New York in their beer (ABC 3§20-d).

The initiative has thus far successfully encouraged the growth of the craft brewing industry. By 2015, less than three years since the Farm Brewery license became available, 106 farm breweries had opened (Office of the Governor Andrew M. Cuomo 2015). On a broader scale, the number of all craft breweries in the state jumped dramatically over the course of four years. There were 92 craft brewers in 2012 (Stonebridge 2015) and 326 in 2016 (New York State Brewers Association 2017), indicating 37% annual growth. Several malt houses have also opened since 2013. Notably, 1886 Malt House, the largest malt house in the state, contracted with 15 farms to start production in 2017. This malt house is owned by Sunoco and has tanks with the capacity to process 60 metric tons of malt at a time, indicating faith in the stability of the

burgeoning industry (Cazentre 2017). Overall, the craft brewing industry supports more than 10,000 jobs, and has an estimated $4.5 billion annual economic impact (New York State Brewers Association 2017), up from an estimated $3.5 billion annual economic impact in 2013 (Stonebridge 2015).

The success demonstrated by the number of new brewers is not entirely reflected in barley production, which was not directly incentivized by the new law (ABC 4§51-a). Historically, New York was an important producer of barley. In the mid-19th century the state grew an estimated two-thirds of the nation's barley (Peterson and Foster 1973). By the turn of the century, the majority of barley production had moved westward (Schwarz 2011). Without a market for barley seed, there was no public or private breeding program focused on developing malting barley for the region. This changed in 2013 when the Cornell Small Grains Breeding Project started to trial existing malting barley varieties and elite breeding lines from around the world, focusing on lines from public breeding programs in Western United States and from seed companies based in Canada and Western Europe (Sorrells et al. 2013a; 2013b). A lack of locally-adapted barley varieties is problematic because the production specifications for malting barley are more stringent than for grains with other end uses. Maltsters require barley to meet several criteria, including a germination of 95% or higher, a protein content between 9.5% and 12.5%, and a low moisture content, to name a few. These specifications are so stringent because low-quality barley does not modify evenly during the malting process and does not have the correct protein and enzymatic composition required for brewing.

Despite the risks, some farmers have started growing the crop to answer the demand for New York-produced malting barley. According to a survey representing 88% of the known malting barley growers in 2015, land planted with malting barley in New York has increased

from 336 acres in 2013 to 875 in 2015 (Newbold and Thayer 2016). In 2017, it was estimated that more than 3,000 acres were planted (Cazentre 2017), suggesting a growth rate of around 75% a year since 2013. At the same time, only 35% of the barley grown met malting grade in 2013 and only 49% met grade in 2014. Even within the scope of research done at Cornell University, trials have run into issues with high winterkill, low germination, and drought. There are still many hurdles to overcome before malting barley can be grown in the state with a relatively low risk of failure. In particular, the surveyed farmers were most concerned about a lack of market demand for their product and the difficulty of growing a crop that meets maltsters' standards (Newbold and Thayer 2016).

One of the risks to barley production is the array of diseases that can reduce yield and quality. This threat is illustrated by the farmer survey conducted in 2015. Growers listed mold and mycotoxins as their biggest grain quality problem (Newbold and Thayer 2016), indicating that farmers are wary of the fungal disease fusarium head blight (FHB) that infects the barley kernels and produces the mycotoxin deoxynivalenol (DON). Barley is screened for DON by maltsters, who will generally reject grain lots with more than 1 ppm of the mycotoxin. FHB is considered to be one of the most economically important diseases in small grains in the United States (Nganje et al. 2004), but it is only one of several fungal diseases that infect barley in New York. The most prevalent and damaging foliar diseases naturally occurring in the state are scald, spot blotch, powdery mildew, and leaf rust (Blachez and Bergstrom 2016a; 2016b; 2017a; 2017b). The research included in this master's thesis revolves around these diseases, the genetic resources available to combat them, and honing the research methods used to study them.

Scald

Scald on barley (*Hordeum vulgare* L.) is a hemibiotrophic fungal disease caused by *Rhynchosporium commune*, which was recently verified to be a distinct species from the rye and triticale pathogen *R. secalis* through phylogenetic analysis (Zaffarano et al. 2011). Up until the past decade, there was uncertainty regarding the host specificity of what was known as *R. secalis*, although it had been noted in the early 20th century that isolates taken from one species tended only to cause disease on the same species (Caldwell 1937). Now it is understood that *R. commune* belongs to a group of closely-related cereal pathogens, each with a relatively narrow host range (Zaffarano et al. 2008, 2011; King et al. 2013). *R. commune* has also been observed to cause disease in various other wild barley (*Hordeum*), brome (*Bromus*), and ryegrass (*Lolium*) species (Zaffarano 2011; King et al. 2013).

Scald is a globally-distributed disease. The fungus causing the disease on rye was originally described in 1897 in Holland and, as early as the 1920s, was noted to have a range including North America, Europe and Australia (Brooks 1928). A decade later, Africa and South America had been added to its recorded range (Caldwell 1937). It is hypothesized that *Rhynchosporium* species became pathogens of barley and rye about 2,500 years ago in Scandinavia, the center of *Rhychosporium* diversity, and then traveled southwards to the Fertile Crescent and Africa (McDonald 2015). Limited genetic diversity in *Rhynchosporium* populations in North America, Australia, and New Zealand indicate that infected seed probably traveled with European colonists to these locations within the past 500 years (Linde et al. 2009).

In a given growing season, initial inoculum of *R. commune* can come from infected seeds or host debris (Caldwell 1937; Jackson and Webster 1976; Fitt et al. 2012). Splash dispersal plays a role in spreading spores either as primary or secondary inoculum (Fitt et al. 1986, 1988).

While spores have been captured from the air using traps, it is unknown how important airborne inoculum is in the development of scald outbreaks (Fitt et al. 2012). After a conidium germinates on a leaf surface, *R. commune* penetrates the epidermis of the host with an appressorium and penetration peg, and initially grows subcutaneously and intracellularly (Caldwell 1937; Lyngs Jørgensen et al. 1993; Zhan 2008). Approximately nine days after infection starts, mesophyll cells in contact with the mycelium collapse and the fungus begins to grow intercellularly. The timing of the mesophyll collapse corresponds with the appearance of water-soaked grayish lesions (Caldwell 1937). Four days later, the lesions dry out, and become first chlorotic and then necrotic (Jackson 1997). The scald lesions are oval to oblong in shape with light gray centers and dark brown edges (Brooks 1928). The lesions are not delimited by the leaf veins, allowing them to appear as large blotches anywhere on the leaf (Jackson 1997). In extreme cases, the disease can completely kill the leaf tissue of the plant (Jackson 1997). It is also possible for *R. commune* to grow within tolerant plants, sporulate, and infect the grain without ever causing symptoms (Atkins et al. 2010). Over the past century, yield losses from scald have been reported as ranging from negligible up to 40% depending on the severity of the infection (Caldwell 1937; Zhan 2008; Fitt et al. 2012). Additionally, plumpness and protein content of the grain can be reduced, damaging the quality of the grain for malting (Khan and Crosbie 1988).

Rhynchosporium spp. are genetically diverse pathogens (Salamati et al. 2000). And, although the teleomorph stages have yet to be discovered, the mating-type genes have been cloned (Foster and Fitt 2003) and the two mating types occur in a one-to-one ratio (Zaffarano et al. 2006), indicating that it is likely that the pathogen can sexually reproduce and undergo sexual recombination. Asexual exchange of genetic material has also been demonstrated in *R. commune* (Forgan et al. 2007). The wide genetic diversity and ability to share and recombine genetic

material suggest that *Rhynchosporium* populations could shift rapidly when exposed to selection pressure from new fungicides or major resistance genes (Zhan 2008). This is supported by populations of *R. commune* naturally developing resistance to benzimidazole (Kendall et al. 1994; Locke and Phillips 1995) and triazole fungicides (Kendall et al. 1993).

Beyond the use of fungicides, growing resistant cultivars is an effective way to control the disease. Resistance to *R. commune* can arise though the barley developing physical barriers to the disease, or through gene-for-gene interactions (Zhan 2008). An example of resistance gained through the development of physical barriers is that barley cultivars resistant to scald are more likely to dramatically modify their cell walls by developing large papillae and haloes around the penetration peg (Jørgensen et al. 1993; Carisse et al. 2000). An example of the classic gene-for-gene interaction occurs between the R-gene, *Rrs1*, in barley, and the avirulence gene, *nip1*, in *R. commune,* that encodes a small phytotoxic protein (Rohe et al. 1995). As would be expected, interactions between barley lines with *Rrs1* and fungal isolates that express the *nip1* gene are incompatible. Overall, at least 17 major resistance gene loci have been described (Wagner et al. 2008). Four of these genes are not currently in domesticated barley, coming instead from wild barley (*H. vulgare* ssp *spontaneum*) and *H. bulbosum* (Wagner et al. 2008). Several quantitative trait loci (QTLs) have been mapped, occurring on all of barley's chromosomes except for 5H (Zhan 2008; Wagner et al. 2008).

Spot blotch

Spot blotch is a globally-distributed foliar disease of wheat, barley, and other grasses. It is caused by the hemibiotrophic fungus, *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*). *B. sorokiniana* can also infect the seeds of its grass hosts, causing black point in the

kernels, and damping off and crown rot in seedlings (Kumar et al. 2002). *B. sorokiniana* is a generalist, and isolates collected from wheat, barley, and rye were all able to cause disease in their original host plant and in the other two hosts (Christensen 1922). The environmental conditions conducive to the development of spot blotch are several days of rain followed by warm (20–30ºC), humid conditions (Kumar et al. 2002; Ghazvini and Tekauz et al. 2007). Yield losses due to spot blotch are unlikely to be catastrophic, but Clark (1979) calculated Ontario's barley yield losses to be 26% and 16% in 1976 and 1977, respectively, due to particularly severe spot blotch epidemics. And, as the disease was emerging in Scotland in the 1970s, some farmers claimed barley yield losses due to spot blotch of more than 50% (Whittle 1977). Spot blotch can be controlled with resistant cultivars, fungicidal applications to the foliage (Clark 1979), and cultural management, such as crop rotation (Duczek et al. 1996; Kumar et al. 2002).

Each year, spot blotch infection can start from infected seed (Henry 1931; Burlakoti et al. 2013) or from inoculum carried off of the other numerous grass hosts of *B. sorokiniana* that often surround wheat or barley fields (Neupane et al. 2010). A single study from Hungary found the fungus causing spot blotch symptoms on 14 wild grass species representing 10 different genera (Bakonyi et al. 1998). During two growing seasons in Canada, the incidence of airborne spores was found to be low before local sources of inoculum, such as dead host tissue, started sporulating (Couture and Sutton 1978). Also, the presence of airborne *B. sorokiniana* spores was correlated with rapidly-declining relative humidity and high windspeeds (Couture and Sutton 1978).

Reports vary regarding how well the pathogen survives on host debris and in the soil. Duczek et al. (1996) found that conidia production on the crowns of crop plants changed from year-to-year, and even varied between cultivars of wheat and barley. The reports of survival in

the soil range from observing no survival of conidia in the soil (Henry 1931; Neupane et al. 2010) to observing good survival of conidia in the soil, especially following the planting of barley or, to a lesser extent, wheat or rye (Chinn et al. 1960; Chinn 1976). In some locations in Canada, soil collected a month after harvest contained more than 5,000 *B. sorokiniana* conidia/g soil (Chinn 1976).

Once a conidium lands on the leaf and a germ tube emerges from the spore, the pathogen invades the host by forming an appressorium, which is used to puncture the cuticle and the cell wall of an epidermal cell (Kumar et al. 2002). The biotrophic growth phase is limited to that epidermal cell, which is completely colonized by hyphae (Kumar et al. 2001). After this point, the necrotrophic stage of the infection begins, and toxins produced by *B. sorokiniana* cause the collapse of mesophyll cells (Kumar et al. 2001).

B. sorokiniana produces several non-host-specific sesquiterpinoid phytotoxins. The first discovered was helminthosporal (De Mayo 1961), which was detected after a cell-free filtrate of the pathogen's culture caused reduction in seedling growth (Ludwig 1957). The toxin inhibits mitochondrial electron transfer and oxidative phosphorylation (Taniguchi and White 1967). Helminthosporol, and its precursor prehelminthosporol (Nilsson et al. 1993), are the most important toxins produced by *B. sorokiniana*. They can disrupt cell membranes, weakening cells prior to invasion, and inhibiting the activity of 1,3-β-glucan synthase, an enzyme involved in the production of callose for plant defense (Briquet et al. 1998; Olbe et al. 1995). Infiltration of host leaves with helminthosporol alone can cause necrotrophic symptoms to develop within 3-4 days (Mercado Vergnes et al. 2006). Additionally, barley cultivars with *mlo* resistance to powdery mildew, a biotrophic pathogen, were found to be more susceptible to spot blotch because the increased accumulation of H_2O_2, caused by the genotype of the barley and the *B. sorokiniana*

toxins, accelerated cell death (Kumar et al. 2001). While programmed cell death (PCD) is an effective defense strategy against powdery mildew, which requires living host tissue to survive, it becomes detrimental in the face of fungi that can survive on dead tissue, like *B. sorokiniana*.

B. sorokiniana is heterothallic, and the perfect form has been produced in the laboratory (Tinline 1951; Zhong and Steffenson 2001a). In nature, the teleomorph and asci have only been observed in Zambia (Kumar et al. 2002). Despite the lack of evidence for sexual recombination in most of the world, there is still a high amount of genetic diversity within *B. sorokiniana* populations, so much so that isolates from the same global region do not always group together in molecular phylogenetic analyses (Zhong and Steffenson 2001b; Mann et al. 2014). It has therefore been hypothesized that *B. sorokiniana* exchanges genetic material through parasexual recombination (Zhong and Steffenson 2001b; Mann et al. 2014). Several pathotypes or virulence groups of *B. sorokiniana* have been determined by screening isolates on differential panels of barley. The first pathotype study was done by Fetch and Steffenson (1994), after lines of two-row barley, previously considered resistant, showed severe susceptibility in 1990. By screening two *B. sorokiniana* isolates on seven barley genotypes, it was determined that the two isolates had different patterns of pathogenicity. Since then, at least four more pathotypes of *B. sorokiniana* have been found in the U.S. and Canada (Ghazvini and Tekauz 2007; Zhong and Steffenson 2001a), three pathotypes have been found in Uruguay (Gamba and Estramil 2002), six have been found in Australia (Meldrum et al. 2004), and three have been found in Syria (Arabi and Jawhar 2004).

There are no major resistance genes for spot blotch in barley, but several studies dedicated to finding QTLs for seedling and adult resistance to spot blotch in barley have been completed. Six-row barley in the midwestern US has maintained resistance to spot blotch for

fifty years, with resistance stemming from a single line, NDB112 (Haas et al. 2016). Zhou and Steffenson (2013) used association mapping in a large population of advanced breeding lines and cultivars to locate the loci responsible for the resistance. They named the set of three loci found on chromosomes 1H, 3H, and 7H the Midwest Six-rowed Durable Resistant Haplotype (MSDRH). Two-row barley does not have the same degree of resistance, despite some lines carrying the three resistance loci of the MSDRH (Zhou and Steffenson 2013).

Powdery Mildew

Powdery mildew on barley is a foliar disease caused by the obligate biotroph, *Blumeria graminis* f. sp. *hordei*. (Bgh) This fungus is closely related to the other *Blumeria graminis formae speciales* that cause powdery mildew on other cereals. Dating back to 1902, powdery mildew of grasses, then called *Erisyphe graminis*, was split into seven 'races' based on which hosts the fungus could infect (Marchal 1902; reviewed in Mains 1933). The most applicable of these to agriculture were the *formae speciales Hordei* on several species within the *Hordeum* genus, *Secalis* on species of the genus *Secale* including rye, *Avenae* on *Avena* species including oats, and *Tritici* infecting *Triticum* species including wheat. In 1985, an eighth *forma specialis*, pathogenic on orchard grass (*Dactylis glomerata*), was identified (Oku et al. 1985; reviewed in Troch et al. 2014). In a multilocus phylogenetic analysis, *B. graminis* f. spp. *tritici*, *secalis*, and *agropyri*, grouped into a single clade, and the isolates of Bgh formed a sister of that clade (Inuma et al. 2007). It also has been well-characterized that the host ranges of the *formae speciales* of *B. graminis* can extend outside of the genera they were named for (Eshmed and Wahl 1970), but those host ranges rarely overlap.

B. graminis overwinters in chasmothecia and spores are dispersed aerially (Johnston 1997). When a *B. graminis* conidium or ascospore lands on a potential host, it adheres to the plant surface by releasing extracellular material with esterases (Nicholson et al. 1988) and cutinases (Pascholati et al. 1992). There is evidence that the plant can sense the extracellular material when an incompatible *forma specialis* produces it on the leaf's surface (Fujita et al. 2004; Zhang et al. 2005). Once attached, the spore forms a primary germ tube, which penetrates the cuticle of the host's cell to absorb water, followed by an appressorial germ tube, an appressorium, and a penetration peg, which pierces through the host's cell wall (Edwards 2002). A successful infection will result in the production of the primary haustorium, a structure that remains within and derives nutrients from the living cell of the host, in the host's epidermal cells (Edwards 2002). The haustorium invaginates rather than punctures the host's cell membrane (Zhang et al. 2005). Non-compatible interactions are characterized by all the same stages of development as those in compatible interactions up to the point of penetration. In non-compatible interactions, the host responds to attempted penetration with formation of papillae, reactive oxygen species (ROS) burst, and hypersensitive response (reviewed in Zhang et al. 2005).

Once infection has started, Bgh produces mycelium, conidia, and eventually chasmothecia on the leaf's surface (Johnston 1997). Bgh also induces the host to accumulate sugars in the infected leaf (Scholes et al.1994), so yield is not only reduced through loss of photosynthetic tissue, but also through the increased respiration of the plant. Smedegaard-Petersen and Stølen (1981) found that the respiration rate of a powdery mildew resistant barley cultivar increased by 80% within 24 hours after inoculation, even if the isolate used was avirulent. Yield losses due to infection with Bgh were 7% using the avirulent isolate and 26% using the virulent isolate (Smedegaard-Petersen and Stølen 1981). On a broader scale, the yield

losses caused by Bgh are moderate to low. Yield losses in barley attributed to powdery mildew have been estimated at about 10% per year in the UK (Johnston 1997), from 5-10% per year in Sweden (Gustafsson and Claesson 1988), and in Australia the estimated loss in value caused by Bgh on a yearly basis is AUD 33 million, or about 2.6% of the crop's annual value (Murray and Brennan 2009). Yield losses in the United States due to powdery mildew are typically less than 1% (Johnston 1997).

The best ways to control powdery mildew are with the use of fungicides and resistant cultivars. The pathogen requires a functioning version of the *Mlo* gene, which codes for an integral membrane protein with still unknown function (Hückelhoven and Panstruga 2011), to be able to successfully colonize the host (Jørgensen and Mortensen 1997). Barley with *mlo*, the non-functional version of the gene, have maintained their effectiveness against powdery mildew since the early 1980s (reviewed in Johnston 1997), but have also been found to be more susceptible to pathogens with a necrotrophic phase such as *B. sorokiniana* (Kumar et al. 2001). There have also been many major resistance genes that have been identified and incorporated into barley, especially on chromosome 1H (Jørgensen 1994). The first race-specific gene to be intentionally incorporated into barley was *Mlg* in the 1930s (Wolfe 1984). The locus with the highest density of major resistant genes is the *Mla* locus with around thirty linked genes and possible allelic variations (Wei et al. 2002). Race-specific, major resistance gene exert strong selection pressure, so they are relatively quickly overcome by Bgh (Wolfe 1984). Researchers have also been working to find quantitative disease resistance that may provide more durable resistance against the pathogen (Backes et al. 2003; Li and Zhou 2011; Spies et al. 2012; Ames et al. 2015).

Leaf Rust

Leaf rust, also known as brown rust, on barley is caused by another obligate biotrophic fungus, *Puccinia hordei*. *P. hordei* is macrocyclic and heteroecious. The uredinia and telia develop on barley and a few closely-related species: *H. vulgare* spp. *spontaneaum*, *H. bulbosum*, and *H. murinum* (Ankister and Wahl 1979). The secondary hosts, observed to be in the genera *Ornithogalum*, *Leopoldia*, and *Dipcadi* (Anikster 1982), are not native to North America. Some, such as Common Star-of-Bethlehem (*Ornithogalum umbellatum*), have been brought in as ornamentals (Mains and Jackson 1924), but are not believed to play a role in the disease cycle in the United States (Johnston 1997b). Without the sexual stage of the fungus, primary inoculum typically comes from over-wintered uredinia or from uredinia that have been carried long-distance aerially (Johnston 1997b). As with *Blumeria graminis*, *Puccinia hordei* forms appressoria and haustoria to invade and obtain nutrients from its hosts (Niks 1983). The pathogen continues the disease cycle by producing uredinia mainly on the upper sides of leaves, but also on the lower sides of leaves, leaf sheaths, and on awns. Telia typically develop later in the season, primarily on leaf sheaths, but also on leaves (Park et al. 2015).

Depending on the cultivar infected, leaf rust can cause a reduction in yield, kernel weight, and protein composition (Newton et al. 1945). In one cultivar tested by Newton et al. (1945), a leaf rust severity of 87.5% resulted in a yield reduction of 23%, while in another variety, a leaf rust severity of 55% did not significantly reduce yield. Similar levels of yield reduction (10-40%) have been found in several experimental trials (Mellville et al. 1976; Arnst et al. 1979; Cotterill et al 1992; Griffey et al. 1994) Leaf rust epidemics starting after barley has reached the milk stage of kernel development (Zadoks 75) are unlikely to significantly reduce yield (Lim and Gaunt 1986). Despite the serious losses due to leaf rust observed in field trials, barley leaf rust

has not been considered to be of significant economic importance in the United States since the 1950s (Griffey et al. 1994). There is a similar situation in Australia, where yearly economic losses due to *Puccinia hordei* have been estimated at AUD 9 million, which is AUD 24 million less than caused by *B. graminis* f.sp. *hordei*, and less than 1% of the total value of the crop (Murray and Brennan 2009). Griffey et al. (1994) also cited the use of resistant cultivars as the main reason barley had not experienced epidemics in recent years, which could change with pathogen evolution.

Fungicidal sprays can be used to manage leaf rust (Melville et al. 1976), but greater attention has been directed towards developing resistant varieties and finding resistance loci. Twenty-one loci conferring hypersensitive seedling resistance have been identified, although seven of these have only been identified in *H. vulgare* ssp. *spontaneum* or *H. bulbosum* (reviewed in Park et al. 2015). Adult plant resistance is determined by partial resistance (Hickey et al. 2011), and only two loci, *Rph20* and *Rph23*, conferring adult plant resistance have been been verified in several environments and designated an *Rph* name (reviewed in Park et al. 2015). Several QTLs have been identified for leaf rust resistance. For example, Spaner et al. (1998) used a biparental mapping population with Harrington and TR306 (a resistant line) to find three QTLs on chromosomes 2H, 5H, and 6H, which collectively explained 45% of the phenotypic variation. While mapping *Rph20* to chromosome 5H, Hickey et al. (2011) also found QTLs on 1H, 3H, 4H, 6H, and 7H. Qi et al. (1999) demonstrated that some of the leaf rust resistance QTLs found in the cultivar Vada were isolate-specific, demonstrating that partial resistance is not necessarily effective against all races of *P. hordei*.

Fusarium head blight

Fusarium head blight (FHB) is a disease of grasses, especially barley and wheat, caused by several *Fusarium* species. It is most commonly caused by *Fusarium graminearum sensu stricto* (teleomorph *Giberella zeae*) in North America (McMullen et al 2012). *F. graminearum sensu stricto,* from here on referred to as *F. graminearum,* is now considered to be only one member of a species complex with at least sixteen species, differentiated using multilocus genotyping and phylogenetic analysis (O'Donnell et al. 2000, 2004, 2008; Starkey et al. 2007). Other species outside of the *Fusarium graminearum* species complex that have been isolated from symptomatic barley grain in New York include *F. sporotrichioides, F. poae, F. tricinctum, F. avenaceum, F. equiseti,* (unpublished) and *F. cerealis* (Cummings et al. 2017a).

Primary inoculum for FHB typically comes from the ascospores formed in perithecia on crop debris, which are induced to forcibly discharge their asci after rainfall and in periods where relative humidity is above 92% (Trail et al. 2002, 2005). Living *F. graminearum* spores have been collected more than 300 meters above the earth's surface, providing evidence that *F. graminearum* spores can be aerially-distributed long distances (Schmale et al. 2012). Once the spores land on the flowering spikelets of their host, they germinate and enter the host through natural openings such as stomata (Pritsch et al. 2000), the base of the lemma and palea, and degraded anther tissues (reviewed in Trail 2009). The fungus grows through the epicarp by entering through the pits and pores of the cell walls into the cytosol (Jansen et al. 2005). The penetrated cells burst or simply die after penetration. Within four days of intercellular growth through the seed, the fungus reaches the endosperm, completely colonizing the seed (Jansen et al. 2015). The secondary metabolite deoxynivalenol (DON) serves as a virulence factor in wheat, the presence of which blocks plant defenses when *F. graminearum* begins its infection of the

rachis. Contrarily, in barley, both a DON-producing and a non-DON-producing mutant were both equally unable to enter the rachis (Jansen et al. 2005). Maier et al. (2006) also found that ability to produce DON did not impact isolates' ability to cause disease in barley, demonstrating that DON is likely not a virulence factor in barley. After colonizing the seed, the pathogen continues its life cycle by producing sporodochia at the surface of the host's infected tissue, ultimately overwintering again as perithecia on plant debris (reviewed in Trail 2009).

In addition to its ability to damage the seeds and the yield of its hosts, FHB is of particular concern because its causal pathogens produce tricothecene mycotoxins, the most common of which is DON. Deoxynivalenol's colloquial name is 'vomitoxin', because it acts as an emetic to some animals that eat the infected grain (Vesonder et al. 1973). Mammals with a single stomach, like pigs, dogs, cats and mice, are most susceptible to the toxin (Vesonder et al. 1973; Hughes et al. 1999; Pestka 2007). While the acute symptoms of exposure are vomiting and other gastrointestinal illness, over the long-term with continuous low dose exposure, mammals can develop symptoms of food avoidance and anorexia (Bergsjø 1992; Prelusky et al. 1994; Pestka 2007). The toxicity of DON is caused by its ability to bind to the 60S subunit of ribosomes, inhibiting translation, and setting off the ribotoxic stress response (Payros et al. 2016). The toxin is not directly regulated by the United States government (USDA 2015), but the industry tends to regulate itself, following the Food and Drug Administration's (FDA) guidelines of not having more than 1 ppm DON in finished wheat products destined for human consumption and not having more than 5 ppm DON on grain products destined to feed for most other animals (FDA 2010). Flour mills typically reject grain lots exceeding 2 ppm, and malt houses will typically reject grain lots exceeding 1 ppm (McMullen et al. 2012).

F. graminearum can also produce toxins other than DON. Deoxynivalenol-producing isolates additionally produce one of two forms of acetylated deoxynivalenol: 3-acetyldeoxynivalenol (3-ADON) or 15-acetyldeoxynivalenol (15-ADON) (Miller et al. 1991). Alternatively, other isolates produce nivalenol (NIV) rather than DON, 3-ADON, or 15-ADON (Ichinoe et al. 1983). Recently, strains of *F. graminearum* that did not produce any of these known toxins were isolated from wheat in Minnesota (Varga et al. 2015). The isolates were found to produce toxins that have now been named NX-2, produced in rice culture, and NX-3, produced predominately in inoculated wheat (Varga et al. 2015). A recent analysis revealed that 20% of 319 isolate of *F. graminearum* collected from wheat spikes, maize ears, maize stubble and the air were NX-2 producers (Lofgren et al. 2017). Additionally, a mechanism for plants to neutralize DON is to metabolize it into deoxynivalenol-3-glucoside (Poppenberger et al. 2003; Lemmens et al. 2005), which, while much less toxic than DON on its own (Pierron et al. 2016), can be cleaved back into DON in the digestive tracts of mammals (Nagle et al. 2014). It has therefore been referred to as a hidden mycotoxin and its importance may be underestimated. There are no FDA guidelines on safe levels of any of these toxins for human consumption.

Fusarium head blight is controlled with cultural methods, fungicides, and resistant cultivars, although no single method is sufficient to control the disease in years where conditions are conducive to development of the disease (McMullen et al. 2008). Cultural management of FHB typically targets initial inoculum produced on crop debris by 1) avoiding planting a host of *F. graminearum* in the same field multiple years in a row and 2) tilling to bury crop residues (Dill-Macky and Jones 2000). Both of these methods are likely to prove ineffective if inoculum is wind-borne into the field (reviewed in McMullen et al. 2012). The most common class of fungicides used to control FHB is the demethylation inhibitor (DMI) triazole fungicides, which

target sterol production in fungi (Paul et al. 2008). The most effective timing depends on the barley cultivar. For chasmogamous, or open-flower type, barley, the optimal spray time is in the middle of anthesis, which is the same as in wheat. Better control of DON production can be achieved with a second spray 17 days after anthesis, when the grain is at the milk stage (Tateishi et al. 2014). Cleistogamous, or closed-flower type, barley lines shed their pollen without opening their florets or extruding their anthers. The anthers may be extruded after pollination has occurred. The best spray timing for cleistogamous lines is when these spent anthers are extruded (Yoshida et al. 2008). There are now forecasting models, based on the weather conditions in the region, to help growers determine whether their crop is at risk for FHB and to better determine when and whether they should spray (De Wolf et al. 2003; De Wolf et al. n.d.)

There are no major resistance genes for FHB known for barley, and it is difficult to breed for FHB resistance in barley because it is a complex trait, strongly influenced by the environment (Capettini et al. 2003). There are no varieties used for resistance on a broad scale. The varieties most commonly used for partial resistance are Chevron for six-rowed varieties, and Harrington, AC Oxbow, and Kitchin for North American two-rowed varieties (Rudd et al. 2001). Mesterhàzy (1995) described five forms of resistance to FHB in wheat, including resistance to I) initial infection, II) spread of infection along the spike, III) decreased kernel size, IV) yield loss, and V) accumulation of mycotoxins. As opposed to wheat, symptoms do not typically spread along the spike in barley, indicating that it has natural Type II resistance (Rudd et al. 2001).

Quantitative trait loci identified for fusarium resistance in barley tend to have small effects, as is expected for a complex, polygenic trait (Massman et al. 2011). Some QTLs that have been found are coincident with loci known to control the physical characteristics of barley, although it has not yet been determined whether this is due to pleiotropy or linkage. For example,

Zhu et al. (1999) found several FHB resistance QTLs across six of the seven barley chromosomes. All but two were colocalized with QTLs for plant development, such as heading date; or physiological traits, such as number of seeds per spike, lateral floret size, inflorescence density, or plant height (Zhu et al. 1999). A recent trancriptomic analysis of lines carrying one of two QTLs identified in Chevron revealed that the QTLs were associated with a heightened defense response in the absence of the pathogen and a more rapid induction of defense responses when infected with the pathogen (Huang et al. 2016)

Currently, researchers are investigating the possibility of controlling FHB through the use of RNA interference (RNAi), using either spray-induced gene silencing (SIGS) or host-induced gene silencing (HIGS). Double-stranded RNA targeting the sterol production sprayed onto barley leaves (Koch et al. 2016) and transgenic wheat expressing RNAi constructs targeting chitin synthesis (Cheng et al. 2015) have both demonstrated the ability to reduce infection by *F. graminearum* (reviewed in Machado et al. 2017).

Fusarium species infecting grain can cause problems during malting and brewing. Malting is the process where grain is germinated in a controlled environment, allowing crucial enzymes to form, and starting the conversion of the stored starches into sugars necessary for fermentation (Mallett 2014). Malting is an unusual post-harvest process in the sense that it requires the seeds to be alive. Since the seeds are alive, any microorganisms inhabiting the seeds also have a good chance of remaining viable during malting (Douglas and Flannigan 1988).

Malting is performed in three main stages: steeping, germination, and kilning (Mallett 2014). Steeping is an approximately 48-hour process where the barley is soaked in water, interrupted with periods of air rests where the water is drained to avoid the build-up of carbon dioxide in the mass of respiring seeds. Steeping serves as the cue to break the dormancy of the

seeds and is followed by germination, where the grain is drained and allowed to start growing (Mallett 2014). During this stage, the grain is mixed at regular intervals, and air is blown through the grain mass to moderate its temperature and moisture content. Germination is allowed to progress for about 96 hours, and is halted by kilning, where the grain is heated to a point where the germinating plants are killed (Mallett 2014).

By micro-malting grain contaminated with FHB pathogens, it has been demonstrated that *F. graminearum* and *F. culmorum* can grow and produce DON during malting of barley (Vegi et al. 2011; Oliviera et al. 2012; Habler et al. 2016) and wheat (Jin et al. 2018). DON largely washes out of the grain during steeping, but germination provides an ideal environment for *F. graminearum* to grow, so DON begins to accumulate again up through the beginning of kilning (Vegi et al. 2011; Oliviera et al. 2012; Habler et al. 2016). The temperature reached during kilning is high enough to kill *F. graminearum*, but DON is a heat-stable molecule (Wolf-Hall et al. 1999; Lauren and Smith 2001; Vidal et al. 2015) so most of the DON produced during malting is carried with the malt into the brewery (Vegi et al. 2011; Habler et al. 2016). This fungal contamination can cause problems in beer because proteins called hydrophobins, produced by *F. graminearum* and other fungi, cause a phenomenon called gushing: the foaming-over of beer that has not otherwise been agitated or heated (Sarlin et al. 2005). And, the DON and other mycotoxins that make it into the beer pose a risk to consumers, as they would in any other food or beverage (Pestka 2010).

Thesis overview

The pathogens reviewed in this chapter have the potential to cause economic loss to growers through yield and quality loss of their barley. The research completed for this thesis was

done with the purpose of finding the best barley varieties to manage these diseases in the state of New York. Chapter 1 is a summary of three years of disease field ratings on winter and spring barley. The goal of the multi-year analysis was to rank cultivars' susceptibility to diseases and to create a resource that would make this information easily accessible to farmers. In chapter 1, spot blotch was identified as a potential risk for spring barley in the state. Therefore, chapter 2 describes a series of experiments designed to determine effective methods for greenhouse spot blotch evaluations. An aggressive isolate from New York was identified, several methods of inoculum production were tested, and twelve seedling and adult cultivars were evaluated for spot blotch susceptibility to choose susceptible and resistant check varieties. The optimal methods identified in chapter 2 were used in chapter 3 to evaluate a two-row spring barley population from the University of Minnesota for susceptibility to spot blotch. Genome-wide association mapping was used to identify QTLs for seedling spot blotch resistance. The same population was planted in the field, and the lines were evaluated as adults for response to spot blotch, powdery mildew, leaf rust, and Fusarium head blight, against natural inoculum. Quantitative trait loci were found for adult resistance to powdery mildew and leaf rust.

CHAPTER 2

SUMMARY OF WINTER AND SPRING VARIETY RESPONSES TO FUNGAL DISEASES IN REGIONAL TRIALS, 2015 - 2017

Introduction

This study explores the possibility of using resistant cultivars of malting barley to manage diseases in New York. The three main ways to control plant diseases are through the use of chemical control, cultural management, and resistant cultivars (Singh 2018). The use of resistant cultivars is particularly attractive because it is less economically and environmentally costly than using pesticides (Lewis et al. 1997). Genetic resistance to disease can be qualitative or quantitative in nature. Qualitative resistance is complete resistance to a disease, conferred by a single gene, and quantitative resistance is partial resistance conferred by several genes with smaller effects (Parlevliet and Zadoks 1977; Poland et al. 2008). It is therefore possible for a set of cultivars to have a range of resistances to the same disease, depending on each cultivar's genetic makeup. Pathogen populations may also differ in their pathogenic characteristics from one region to another (McDermott and McDonald 1993), so cultivars that have been determined to have resistance to a disease in one area may not have the same level of resistance when challenged by the natural inoculum in another region. To determine whether a cultivar is resistant to a disease in a region, it is best to screen it in a range of environments over several years.

For farmers interested in selling their barley to malt houses, disease-resistant malting barley cultivars must also have good agronomic and malting characteristics to be a viable option. Malting barley has not been grown in New York to any appreciable degree since the beginning

of the 20th century (Schwarz 2011), so there are no barley cultivars that have been bred and selected specifically for use in New York. It takes about a decade to cross, inbreed, trial, and increase the seed of potential new barley cultivars. Therefore, to be able to respond quickly to demand for barley seed following the creation of the Farm Brewery License, it was logical to test existing cultivars adapted to climates similar to New York's.

Cornell's Small Grains Breeding Project began to perform such trials in 2013 (Sorrells et al. 2013a; 2013b). To test a range of possible barley options for growers, these trials included two- and six-row winter and spring barley varieties. The barley varieties came from a range of environments, including Washington State, Idaho, Minnesota, Canada, and western Europe. The trials were planted specifically to collect agronomic data, such as yield, test weight, and lodging; and to collect malt quality data, such as levels of malt extract, protein, and beta-glucans. Adventitiously, the lack of fungicide treatment also allowed for the collection of data on naturally-occurring diseases. Disease ratings were taken over the course of three growing seasons, from 2015-17.

The purpose of this study was to use the disease data collected from 2015-17 to 1) determine which diseases pose a threat to barley cultivation in the state of New York, and to 2) rank agronomically-promising cultivars in terms of resistance to these diseases. *Note: a more complete year-by-year summary of the ratings, including all of the lines trialed, can be found in the six Plant Disease Management Reports in the Appendix.*

Methods

Trials and locations

Each year, winter barley variety trials and spring barley variety trials were performed at several locations in New York (Tables 1 and 2). The most-replicated trials were the Winter Malting Barley Trial (WMBT) and the Spring Malting Barley Trial (SMBT), each of which were planted in four locations every year: two in Ithaca, (Tompkins Co.) NY and two in locations farther to the north and west. Several farms in Ithaca were used for variety trials every year, including Helfer, Ketola, Snyder, and McGowan, each with unique microenvironment and cropping history. Locations for the regional trials outside of Ithaca were in Monroe, Seneca, Genesee, and Steuben Counties. Other trials rated for disease were the Winter Barley Germplasm Nursery (WBGN), the Winter Malting Barley Coop (WintMaltCoop), and the Eastern Spring Barley Nursery (ESBN) all of which were only planted at locations near Ithaca, NY (Tables 1 and 2).

Experimental design and field preparation

Each trial was laid out in a randomized complete block design, blocked by replicate. There were three replications at each field site, and two- and six-row varieties were completely randomized within each replicate. Plots were 4 m long and 6 rows wide with 18 cm row spacing. Seed was sown at a rate of 107.6 kg/ha. The winter trials were planted in late September or early October of the preceding year (Table 1). The spring trials were planted in late April or early May (Table 2). In 2015 and 2016, winter barley fields were prepared with 224 kg/ha of a 10:20:20 fertilizer before planting, delivering 22.4 kg/ha of nitrogen. In 2017, the winter barley fields were prepared with an application of 22.4 kg/ha of 20:20:20, delivering 44.8 kg/ha of nitrogen. The winter barley fields were also topdressed with 56 kg/ha of nitrogen in the spring, except for 2016

when no additional nitrogen was applied. In 2015 and 2016, spring trials were prepared with a 336 kg/ha application of 10:20:20, delivering 33.6 kg/ha of nitrogen. In 2017, the spring barley fields were prepared with a 134.5 kg/ha application of 27:18:9 fertilizer, delivering 35.9 kg/ha of nitrogen. In the spring, all trials were sprayed with broadleaf herbicides. In 2015 and 2016, a mixture of Maestro 2EC (bromoxynil, Nufarm, Melbourne, Australia) and Harmony Extra SG (a mixture of thifensulfuron methyl and tribenuron methyl, DuPont, Newark, DE) with Induce (nonionic surfactant, Helena Chemical, Collierville, TN) was applied. In 2017, a mixture of herbicides and adjuvant containing the same active ingredients as were used in the previous two years was applied. No fungicides or artificial disease inoculations were applied.

Table 1. Year, location, trial name, planting dates, and rating dates of winter barley trials with adequate disease data to be included in the analysis.

Year	Location	Trial[y]	Date Planted	Date Foliar Diseases Were Rated	Growth Stage at Foliar Disease Rating	Date FHB[z] Was Rated	Growth Stage at FHB Rating
2015	Tompkins-Helfer	WMBT	25-Sep-14	20-Jun-15	Hard Dough	20-Jun-15	Hard Dough
	Tompkins-Ketola	WMBT	30-Sep-14	19-Jun-15	Hard Dough	19-Jun-15	Hard Dough
2016	Tompkins-Ketola	WMBT	5-Oct-15	1-Jun-16	Hard Dough	NA	NA
	Tompkins-Snyder	WMBT	23-Sep-15	1-Jun-16	Hard Dough	NA	NA
2017	Tompkins-Ketola	WMBT	6-Oct-16	2-Jun-17	Soft Dough	17-Jun-17	Ripening
		WBGN	6-Oct-16	2-Jun-17	Flowering	20-Jun-17	Ripening
	Tompkins-McGowan	WMBT	28-Sep-16	2-Jun-17	Soft Dough	20-Jun-17	Ripening
		WintMaltCoop	28-Sep-16	10-Jun-17	Hard Dough	17-Jun-17	Ripening
	Monroe	WMBT	27-Sep-16	18-Jun-17	Hard Dough	18-Jun-17	Hard Dough
	Seneca	WMBT	7-Oct-16	5-Jun-17	Soft Dough	18-Jun-17	Hard Dough

[y] WMBT = Winter Malting Barley Trial, WBGN= Winter Barley Germplasm Nursery, WintMaltCoop = Winter Malting Barley Coop,
[z] FHB =Fusarium head blight

Table 2. Year, location, trial name, planting dates, and rating dates of spring barley trials with adequate disease data to be included in the analysis.

Year	Location	Trial[y]	Date Planted	Date Foliar Diseases Were Rated	Growth Stage at Foliar Disease Rating	Date FHB[z] Was Rated	Growth Stage at FHB Rating
2015	Genesee	SMBT	11-May-15	17-Jul-15	Hard Dough	17-Jul-15	Hard Dough
	Tompkins-Ketola	SMBT	5-May-15	7-Jul-15	Soft Dough	15-Jul-15	Hard Dough
	Tompkins-Snyder	SMBT	28-Apr-15	3-Jul-15	Soft Dough	8-Jul-15	Hard Dough
	Steuben	SMBT	29-Apr-15	17-Jul-15	Hard Dough	17-Jul-15	Hard Dough
2016	Tompkins-Ketola	SMBT	20-Apr-16	8-Jul-16	Hard Dough	8-Jul-16	Hard Dough
	Tompkins-Helfer	SMBT	28-Apr-16	18-Jul-16	Hard Dough	18-Jul-16	Hard Dough
2017	Tompkins-Helfer	SMBT	28-Apr-17	14-Jul-16	Hard Dough	NA	NA
		ESBN	28-Apr-17	17-Jul-16	Hard Dough	NA	NA
	Tompkins-Ketola	SMBT	24-Apr-17	15-Jul-16	Hard Dough	NA	NA
		ESBN	24-Apr-17	15-Jul-16	Hard Dough	NA	NA
	Genesee	SMBT	11-May-17	16-Jul-16	Hard Dough	NA	NA
	Steuben	SMBT	27-Apr-17	16-Jul-16	Hard Dough	NA	NA

[y] SMBT = Spring Malting Barley Trial, ESBN = Eastern Spring Barley Nursery
[z] FHB =Fusarium head blight

Varieties

Each trial included at least 20 cultivars and breeding lines, but not all varieties were included in every trial. Even the WMBT and SMBT had varieties removed and added on a yearly basis. To maintain a balanced statistical design, the varieties that had data for all three years in the WMBT and SMBT were selected for analysis (Table 4 and 5). The cultivar Wintmalt, was included in the WMBT in 2015 and 2016, but was dropped in 2017. It was instead included in the WBGN and WintMaltCoop in 2017, along with the WMBT varieties Charles and Endeavor (Table 4). This allowed all varieties except for Wintmalt to be statistically analyzed together, and allowed Wintmalt to be analyzed separately with Charles and Endeavor. The results for the two separate analyses could subsequently be compared. A description of the varieties included in the

winter malting barley analysis is summarized in Table 3. Which varieties were included in each trial is summarized in Table 4.

Table 3. Description of varieties included in the winter barley analysis.

Variety	Rows	Type	Variety's Developer
Charles	2	Malting	USDA-ARS, Aberdeen, ID
Endeavor	2	Malting	USDA-ARS, Aberdeen, ID
Flavia	2	Malting	Ackermann Saatzucht, Germany
KWS Scala	2	Malting	KWS, Europe
Nectaria	2	Malting	Secobra, France
Saturn	6	Feed	Limagrain, Europe
SY Tepee	2	Malting	Syngenta
Wintmalt	2	Malting	KWS, Europe

Table 4. Varieties included in winter barley trials.

			Varieties							
Year	Location	Trial	Charles	Endeavor	Flavia	KWS Scala	Nectaria	Saturn	SY Tepee	Wintmalt
2015	Tompkins-Helfer	WMBT	X	X	X	X	X	X	X	X
	Tompkins-Ketola	WMBT	X	X	X	X	X	X	X	X
2016	Tompkins-Ketola	WMBT	X	X	X	X	X	X	X	X
	Tompkins-Snyder	WMBT	X	X	X	X	X	X	X	X
2017	Tompkins-Ketola	WMBT	X	X	X	X	X	X	X	
		WBGN	X	X						X
	Tompkins-McGowan	WMBT	X	X	X	X	X	X	X	
		WintMaltCoop	X	X						X
	Monroe	WMBT	X	X	X	X	X	X	X	
	Seneca	WMBT	X	X	X	X	X	X	X	

[y] WMBT = Winter Malting Barley Trial, WBGN= Winter Barley Germplasm Nursery, WintMaltCoop = Winter Malting Barley Coop

There were twelve spring barley varieties included in all three years of the SMBT. A selection of these was also included in the 2017 ESBN (Table 5). It was therefore possible to complete one analysis with all twelve varieties, and then complete a second, more robust analysis with only the seven varieties included in both the SMBT and the ESBN.

Table 5. Description of the spring barley varieties analyzed and in which trials they were included (SMBT only vs. SMBT and ESBN)

Varieties	Rows	Type	Variety's Developer	Included in SMBT[y]	Included in ESBN[z]
AAC Synergy	2	Malting	Agriculture and Agri-Food Canada	X	X
Bastile	6	Feed	Synagri, Canada	X	
Cerveza	2	Malting	Agriculture and Agri-Food Canada	X	
Conlon	2	Malting	North Dakota State University	X	X
Craft	2	Malting	Montana State University	X	
Harmony	6	Feed	Synagri, Canada	X	
KWS Tinka	2	Malting	KWS, Europe	X	X
ND Genesis	2	Malting	North Dakota State University	X	X
Newdale	2	Malting	Agriculture and Agri-Food Canada	X	X
Oceanik	6	Feed	Synagri, Canada	X	
Pinnacle	2	Malting	North Dakota State University	X	X
Quest	6	Malting	University of Minnesota	X	X

[y] Spring Malting Barley Trial
[z] Eastern Spring Barley Nursery

Disease rating

The foliar diseases rated include scald (*Rhynchosporium commune*), spot blotch (*Bipolaris sorokiniana*), leaf rust (*Puccinia hordei*), and powdery mildew (*Blumeria graminis* f.sp. *hordei*). The only disease rated on the spike was Fusarium head blight (FHB), caused primarily by *F. graminearum.* Identity of the diseases was determined by observing the symptoms and signs on the host, and by morphologically identifying the fungal spores and structures using light microscopy.

Foliar disease severities were estimated as the percent of the top two leaves affected by the disease, i.e. showing symptoms or signs of the disease, over the entire plot. Fusarium head blight was scored by measuring the incidence and severity of the disease in each plot and calculating the FHB index. Incidence was estimated by looking at 25 heads, counting how many were symptomatic, and multiplying by four. Severity of FHB in each plot was determined by estimating the average percentage of kernels with the disease on the symptomatic heads, reported as a whole number. The FHB index was calculated by multiplying incidence by severity and

dividing by 100. The diseases were rated at their approximate peak severity before the barley senesced (Tables 1 and 2).

Since the diseases were naturally-occurring, not all diseases developed to ratable levels at all locations in all years. Only those trials with more than trace amounts of the disease were included for analysis. A summary of which trials were used for the analyses of which diseases are summarized in Tables 6-9.

Table 6. Winter barley trials used for the analysis of each disease, excluding the Wintmalt cultivar.

Year	Location	Trial[y]	Scald	Leaf Rust	Powdery Mildew	FHB[z]
2015	Tompkins-Helfer	WMBT				X
	Tompkins-Ketola	WMBT	X	X		X
2016	Tompkins-Ketola	WMBT	X		X	
	Tompkins-Snyder	WMBT	X	X	X	
2017	Tompkins-Ketola	WMBT	X		X	X
		WBGN				
	Tompkins-McGowan	WMBT	X		X	X
		WintMaltCoop				
	Monroe	WMBT	X	X		X
	Seneca	WMBT	X			X

[y] WMBT = Winter Malting Barley Trial, WBGN= Winter Barley Germplasm Nursery, WintMaltCoop = Winter Malting Barley Coop
[z] FHB = Fusarium head blight

Table 7. Winter barley trials used for the analysis of each disease, including the Wintmalt cultivar.

Year	Location	Trial[y]	Scald	Leaf Rust	Powdery Mildew	FHB[z]
2015	Tompkins-Helfer	WMBT				X
	Tompkins-Ketola	WMBT		X		X
2016	Tompkins-Ketola	WMBT	X	X		
	Tompkins-Snyder	WMBT	X	X		
2017	Tompkins-Ketola	WMBT				
		WBGN	X			X
	Tompkins-McGowan	WMBT				
		WintMaltCoop	X	X		X
	Monroe	WMBT				
	Seneca	WMBT				

[y] WMBT = Winter Malting Barley Trial, WBGN= Winter Barley Germplasm Nursery, WintMaltCoop = Winter Malting Barley Coop
[z] FHB = Fusarium head blight

Table 8. Spring barley trials (SMBT only) used in the analysis of each disease.

Year	Location	Trial[y]	Leaf Rust	Powdery Mildew	Spot Blotch	FHB[z]
2015	Genesee	SMBT			X	X
	Tompkins-Ketola	SMBT	X	X	X	X
	Tompkins-Snyder	SMBT			X	X
	Steuben	SMBT		X	X	X
2016	Tompkins-Ketola	SMBT	X			
	Tompkins-Helfer	SMBT	X			
2017	Tompkins-Helfer	SMBT		X	X	
		ESBN				
	Tompkins-Ketola	SMBT	X	X		
		ESBN				
	Genesee	SMBT		X		
	Steuben	SMBT	X	X	X	

[y] SMBT = Spring Malting Barley Trial, ESBN = Eastern Spring Barley Nursery
[z] FHB =Fusarium head blight

Table 9. Spring barley trials (SMBT and ESBN) used in the analysis of each disease.

Year	Location	Trial[y]	Leaf Rust	Powdery Mildew	Spot Blotch
2015	Genesee	SMBT			X
	Tompkins-Ketola	SMBT		X	X
	Tompkins-Snyder	SMBT			X
	Steuben	SMBT		X	X
2016	Tompkins-Ketola	SMBT	X		
	Tompkins-Helfer	SMBT	X		
2017	Tompkins-Helfer	SMBT		X	X
		ESBN	X	X	X
	Tompkins-Ketola	SMBT	X	X	
		ESBN	X	X	X
	Genesee	SMBT		X	
	Steuben	SMBT	X	X	X

[y] SMBT = Spring Malting Barley Trial, ESBN = Eastern Spring Barley Nursery

Statistical analysis

Ratings, and therefore experimental variance, from the same location evaluated in different years would likely be correlated because the location shares its disease inoculum sources and will have a similar microclimate to itself year after year, which violates the

independence of errors assumption of analysis of variance. The same replicate from the same field location in different years were therefore averaged to collapse the location by year effects into a single set of values for each location (Snedecor and Cochran 1967). In some cases, multiple trials were planted in the same location in the same year. These values were also pooled with the rest of the data from the same location. For each disease analyzed, only those trials with more than trace quantities of the disease were included in the analysis. In particular, Wintmalt was not included in the analysis for powdery mildew because none of the trials in which it was included had high enough powdery mildew disease to statistically analyze. To obtain normally-distributed data, all disease ratings were square root transformed before analysis. ANOVA was performed with variety and location as main effects, and location × variety as an interaction effect. The means of the transformed data for each variety were separated by Tukey's Honest Significant Difference ($\alpha = 0.05$).

Results

Diseases on Winter Barley

Scald

The analysis of the scald severities for all cultivars, excluding Wintmalt, included seven trials, with at least one in all three years (Table 6). The analysis resulted in a separation of varieties into three statistically significant groups (Table 10). All effects included in the ANOVA were found to be highly significant (Table 11). KWS Scala was the most susceptible cultivar, reaching a maximum severity of 75% in Monroe County in 2017. Flavia was also quite susceptible, reaching a severity of 70% in the same location. Charles had an overall average of 13.8% severity, around an order of magnitude greater than the more resistant varieties, indicating

that it is at least moderately susceptible to scald. The remaining varieties never reached a severity greater than 5% at any location (Table 10), providing strong evidence that they are currently at least moderately resistant to New York populations of *R. commune* to which they have been exposed.

Table 10. Mean scald severities separated for winter malting barley varieties and the means of the two trials with the highest levels of scald severity.

Variety		Overall average (N = 21 plots)	Percent severity at the trial with the most scald (Monroe 2017)	Percent severity at the trial with the second-highest level of scald (Tompkins-Ketola 2017)
KWS Scala	a[x]	26.5	75.0	60.0
Flavia	ab	19.3	70.0	46.7
Charles	b	13.8	3.3	30.0
Endeavor	c	1.8	3.7	3.0
SY Tepee	c	1.6	5.0	2.2
Nectaria	c	0.3	0	2.0
Saturn	c	0.1	0	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 11. Significance of main effects and the interaction term from the ANOVA for the transformed scald severity data without Wintmalt.

Effect	p-value	Level of significance[k]
Variety	1.90E-15	***
Location	2.39E-04	***
Variety × Location	3.78E-07	***

[k] n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p<0.001$

The analysis to determine Wintmalt's susceptibility to scald was performed on four locations planted in either 2016 or 2017 (Table 7). The ANOVA yielded non-significant results (Tables 12 and 13), but the consistent ordering of Wintmalt as the most susceptible variety (Table 12) indicates that this was likely due to a lack of power in the analysis, rather than a lack

of differentiation between the cultivars in terms of resistance to scald. There is therefore observational evidence that Wintmalt is at least moderately susceptible to scald.

Table 12. Mean scald severities for Wintmalt, Charles, and Endeavor, and the means of the two trials with the highest levels of scald severity.

Variety		Overall Average (N = 12 plots, 4 trials)	Percent severity at the location with the most scald (Tompkins-Ketola 2017)	Percent severity at the location with the second-highest level of scald (Tompkins-McGowan 2017)
Wintmalt	a[x]	28.0	60.0	45.0
Charles	a	20.5	27.0	33.3
Endeavor	a	3.0	8.3	3.3

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 13. Significance of main effects and the interaction term from the ANOVA run on the transformed scald severity data with Wintmalt, Charles, and Endeavor.

Effect	p-value	Level of significance[k]
Variety	0.08	n.s.
Location	0.57	n.s.
Variety × Location	0.62	n.s.

[k] n.s. = not significant, * = p < 0.05,
** = p < 0.01, *** = p<0.001

Powdery mildew

Powdery mildew never reached the same level of severity as scald did in the winter barley trials. The maximum level of powdery mildew observed was 35% (Table 14), whereas the highest level of scald observed was 75% (Table 10). Even so, the statistical analysis of the winter barley cultivars yielded highly significant differences in susceptibility to powdery mildew (Table 15). The varieties resolved into three distinct groups (Table 14). Nectaria was always the most susceptible. Endeavor was the only other variety whose average severity was statistically different from 0%, although severity on Endeavor never exceeded 1%. Without adequate

powdery mildew on Wintmalt, Endeavor, or Charles in any trial where all three were included, it was not possible to determine Wintmalt's susceptibility ranking.

Table 14. Mean powdery mildew severities separated for winter malting barley varieties and the means of the two trials with the highest levels of powdery mildew severity.

Variety		Overall Average (N = 12 plots per variety)	Percent severity at the trial with the highest level of powdery mildew (Tompkins-McGowan 2017)	Percent severity at the trial with the second-highest level of powdery mildew (Tompkins-Snyder 2016)
Nectaria	a[x]	11.5	35.00	5.00
Endeavor	b	0.29	0.83	0.33
Flavia	bc	0.17	0.50	0
KWS Scala	bc	0.04	0.17	0
Saturn	bc	0.04	0.17	0
Charles	c	0	0	0
SY Tepee	c	0	0	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 15. Significance of main effects and the interaction term from the ANOVA run on the transformed powdery mildew severity data.

Effect	p-value	Level of significance[k]
Variety	< 2.2E-16	***
Location	1.94E-13	***
Variety × Location	2.32E-16	***

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Leaf rust

The analysis for leaf rust included three trials spanning all three years of the study (Table 6). Variety was highly significant in the ANOVA (Table 17), and the varieties resolved into two clear groupings, with a possible third intermediary group (Table 16). Charles was the most susceptible variety, reaching a 23.7% severity in the WMBT in Monroe County in 2017 (Table 16) and reaching a 36.7% severity on the WintMaltCoop trial in Tompkins-McGowan in 2017 (Table 18). Saturn demonstrated some level of susceptibility, reaching a leaf rust severity of

8.8% in Monroe in 2017 (Table 18). All other varieties had leaf rust severities under 3% at all locations.

Table 16. Mean leaf rust severities separated for winter malting barley varieties and the means of the two trials with the highest levels of leaf rust severity.

Variety		Overall Average (N = 9 plots per variety)	Percent severity at the trial with the most leaf rust (Monroe 2017)	Percent severity at the trial with the second-highest level of leaf rust (Tompkins-Ketola 2015)
Charles	a[x]	14.9	23.7	12.7
Saturn	ab	3.7	8.8	1.3
Endeavor	b	1.1	0.3	2.7
Flavia	b	0.3	0.3	0.5
Nectaria	b	0.4	0.5	0.5
SY Tepee	b	0.2	0.2	0.5
KWS Scala	b	0.1	0	0.2

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 17. Significance of main effects and the interaction term from the ANOVA run on the transformed leaf rust severity data.

Effect	p-value	Level of significance[k]
Variety	2.81E-04	***
Location	2.38E-01	n.s.
Variety × Location	9.11E-01	n.s.

[k] n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p<0.001$

As with scald, the leaf rust analysis of Wintmalt, Charles, and Endeavor, did not yield statistically significant results (Table 18 and 19). Even so, Wintmalt had an overall average leaf rust severity ten times less than the susceptible Charles (Table 18), providing observational evidence that it is moderately resistant to leaf rust.

Table 18. Mean leaf rust severities for Wintmalt, Charles, and Endeavor and the means of the two trials with the highest levels of leaf rust severity.

Variety		Overall Average (N = 12 plots per variety)	Percent severity at the trial with the most leaf rust (Tompkins-McGowan 2017)	Percent severity at the trial with the second-highest level of leaf rust (Tompkins-Snyder 2016)
Charles	a[x]	17.9	36.7	21.7
Endeavor	a	1.8	2.2	2.0
Wintmalt	a	1.7	0.5	5.2

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 19. Significance of main effects and the interaction term from the ANOVA run on the transformed leaf rust severity data with Wintmalt, Charles, and Endeavor.

Effect	p-value	Level of significance[k]
Variety	0.08	n.s.
Location	0.65	n.s.
Variety × Location	0.76	n.s.

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Fusarium head blight

Six trials that took place in 2015 and 2017 were included in the analysis of Fusarium head blight (Table 6). From the ANOVA, variety was determined to be significant in explaining the variance in FHB index (Table 21). The varieties resolved into two distinct groups, with one possible intermediary group (Table 20). Flavia stood out as the variety with the highest degree of susceptibility to FHB, reaching a peak index of 7.34 in Tompkins-Ketola in 2015 (Table 20). Endeavor was the least susceptible variety. Its overall average index was 0.28, which does not indicate full resistance to the disease. All other varieties could not be statistically separated from either Flavia or Endeavor.

Table 20. Mean FHB indexes separated for winter malting barley varieties and the means of the two trials with the highest levels of leaf rust severity.

Variety		Overall Average (N = 18 plots per variety)	FHB Index at the trial with the most FHB (Tompkins-Helfer 2015)	FHB Index at the trial with the second-highest level of FHB (Tompkins-Ketola 2015)
Flavia	a[x]	1.32	5.09	7.34
Charles	ab	1.05	2.60	5.27
Saturn	ab	1.00	3.13	5.43
Nectaria	ab	0.83	3.96	1.07
SY Tepee	ab	0.76	5.72	1.63
KWS Scala	ab	0.33	4.70	0.83
Endeavor	b	0.28	1.43	1.09

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 21. Significance of main effects and the interaction term from the ANOVA run on the transformed FHB index data

Effect	p-value	Level of significance[k]
Variety	0.027	*
Location	< 2.2E-16	***
Variety × Location	1.52E-03	**

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Analysis of FHB index for Wintmalt, Charles, and Endeavor also showed variety to be significant (Table 23). Wintmalt ranked as the variety with the highest level of FHB, but was not statistically different from Charles (Table 22). Endeavor again ranked as the least susceptible variety to FHB (Table 22).

Table 22. Mean FHB indexes separated for Wintmalt, Charles, and Endeavor and the means of the two trials with the highest levels of Fusarium head blight

Variety		Overall Average (N = 12 plots per variety)	FHB Index at the location with the highest level of FHB (Tompkins-Helfer 2015)	FHB Index at the location with the second-highest level of FHB (Tompkins-Ketola 2015)
Wintmalt	a[x]	1.00	4.74	1.93
Charles	ab	1.50	2.60	5.27
Endeavor	b	0.40	1.40	1.09

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 23. Significance of main effects and the interaction term from the ANOVA run on the transformed FHB index data with Wintmalt, Charles, and Endeavor.

Effect	p-value	Level of significance[k]
Variety	0.011	*
Location	3.53E-06	***
Variety × Location	0.100	n.s.

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Diseases on spring barley

Spot blotch

For the spring barley spot blotch analysis, data were collected from all four SMBT locations in 2015 and two of the locations from 2017, for a total of six trials (Table 8). For the varieties included in both the SMBT and the ESBN, an additional two trials in 2017 were used in the second analysis (Table 9). In both ANOVAs, variety was found to be highly significant (Tables 25 and 27). Also, the overall ranking of the varieties remained the same between the two analyses (Tables 24 and 26). Pinnacle was the most susceptible variety overall, but it was overtaken or closely matched by KWS Tinka in Tompkins-Helfer 2017, depending on whether the SMBT or ESBN was considered (Table 26). In other locations such as Steuben and Tompkins-Ketola in 2017, KWS Tinka had spot blotch severities that were lower than Pinnacle's

(Tables 24 and 26). This observation of a variety by location interaction was supported by both ANOVAs (Tables 25 and 27)

The high ratings at Tompkins-Helfer in 2017 demonstrated that cultivars could have moderate susceptibility to spot blotch under high disease pressure, despite showing low- to no susceptibility under other conditions. For example, Newdale had no disease in either Tompkins-Ketola or Steuben in 2017, but had around 15% severity across the SMBT and ESBN in Tompkins-Helfer in 2017 (Table 26).

The varieties that had less than 10% spot blotch severity in all trials include Craft, Oceanik, Quest, AAC Synergy, Harmony, and Bastile. Of these, only Craft, Quest, and AAC Synergy are malting varieties.

Table 24. Mean spot blotch severities separated for SMBT varieties and the means of the two trials with the highest levels of spot blotch severity.

Variety		Overall Average (N = 18 plots per variety)	Percent severity at the trial with the highest level of spot blotch (Tompkins-Helfer 2017)	Percent severity at the trial with the second-highest level of spot blotch (Steuben 2017)
Pinnacle	a[x]	17.6	60.0	26.8
KWS Tinka	ab	15.2	80.0	3.7
ND Genesis	bcd	6.8	33.3	3.7
Conlon	bc	5.8	23.3	1.7
Newdale	cde	3.4	18.3	0
Cerveza	cde	3.0	15.0	0.3
Craft	cde	1.8	5.0	0
Oceanik	cde	1.4	2.3	0.7
Quest	de	1.0	0	0
AAC Synergy	e	0.9	3.7	0
Harmony	e	0.7	2.3	0
Bastile	e	0.5	0	0.5

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 25. Significance of main effects and the interaction term from the ANOVA run on the transformed spot blotch severities of the SMBT varieties.

Effect	p-value	Level of significance[k]
Variety	<2.2E-16	***
Location	<2.2E-16	***
Variety × Location	5.35E-12	***

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Table 26. Mean spot blotch severities separated for SMBT and ESBN varieties and the means of the two locations with the highest levels of spot blotch severity.

Variety		Overall Average (N = 23 plots per variety)	Percent severity at the location with the highest level of spot blotch (Tompkins-Helfer 2017)	Percent severity at the trial with the second-highest level of spot blotch (Tompkins-Ketola 2017)
Pinnacle	a[x]	34.2	80.0	85.0
KWS Tinka	ab	21.9	76.7	5.0
ND Genesis	bc	11.5	39.2	3.0
Conlon	c	9.1	29.2	0
Newdale	cd	4.4	15.8	0
AAC Synergy	d	1.6	5.2	0.5
Quest	d	0.8	0.2	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 27. Significance of main effects and the interaction term from the ANOVA run on the transformed spot blotch severities of the SMBT and ESBN varieties.

Effect	p-value	Level of significance[k]
Variety	<2.2E-16	***
Location	<2.2E-16	***
Variety × Location	9.71E-10	***

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Powdery mildew

For the spring barley powdery mildew analysis, data were used from two of the four SMBT locations in 2015 and all four of the locations in 2017, for a total of six trials (Table 8). For the varieties included in both the SMBT and the ESBN, both ESBN trials from 2017 were added for a second analysis (Table 9). In both ANOVAs, variety was found to be highly significant (Tables 29 and 31). Only KWS Tinka and Conlon swapped ranking order between the two analyses (Tables 28 and 30). The varieties were separated into three distinct groups for the

varieties in only in the SMBT, and into two groups for the varieties included in both the SMBT and ESBN (Tables 28 and 30).

Quest was the only highly susceptible variety. Craft also showed some susceptibility, regularly reaching a severity half of Quest's (Table 28). All the other varieties grouped together, and could be considered resistant, although Pinnacle and Oceanik had powdery mildew severities above 10% in Steuben in 2017 (Tables 28 and 29).

Table 28. Mean powdery mildew severities separated for SMBT varieties and the means of the two trials with the highest powdery mildew severity.

Variety		Overall Average (N = 17 plots per variety)	Percent severity at the trial with the highest level of powdery mildew (Steuben 2017)	Percent severity at the trial with the second-highest level of powdery mildew (Tompkins-Helfer 2017)
Quest	a[x]	46.3	85.0	68.3
Craft	b	20.2	46.7	26.7
Oceanik	c	8.5	16.8	0.5
Pinnacle	c	4.5	11.7	5.0
AAC Synergy	c	3.0	6.8	0.5
Cerveza	c	2.5	6.8	0
ND Genesis	c	2.2	0	1.7
Newdale	c	1.9	2.2	0
Harmony	c	0.9	1.8	0
KWS Tinka	c	0.6	0	0
Bastile	c	0.5	1.7	0
Conlon	c	0.4	1.7	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 29. Significance of main effects and the interaction term from the ANOVA run on the transformed powdery mildew severities of the SMBT varieties.

Effect	p-value	Level of significance[k]
Variety	<2.2E-16	***
Location	6.70E-05	***
Variety × Location	0.0007131	***

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Table 30. Mean powdery mildew severities separated for SMBT and ESBN varieties and the means of the two trials with the highest levels of powdery mildew severity.

Variety		Overall Average (N = 22 plots per variety)	Percent severity at the trial with the highest level of powdery mildew (Steuben 2017)	Percent severity at the trial with the second-highest level of powdery mildew (Tompkins-Helfer 2017)
Quest	a[x]	44.6	85.0	68.3
Pinnacle	b	3.5	11.7	5.0
AAC Synergy	b	2.8	6.8	0.5
ND Genesis	b	2.6	0	1.7
Newdale	b	1.7	2.2	0
Conlon	b	1.7	1.7	0
KWS Tinka	b	0.5	0	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 31. Significance of main effects and the interaction term from the ANOVA run on the transformed powdery mildew severities of the SMBT and ESBN varieties.

Effect	p-value	Level of significance[k]
Variety	<2.2E-16	***
Location	5.46E-05	***
Variety × Location	0.002	**

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Leaf rust

The spring barley leaf rust analysis included data from one SMBT trial in 2015, and two each in 2016 and 2017, for a total of 5 trials (Table 8). For the varieties included in the SMBT and the ESBN, the two ESBN trials from 2017 were added for the second analysis (Table 9). In both ANOVAs, variety was found to be significant (Tables 33 and 35). In the two analyses, the varieties resolved into two groups, with many varieties of intermediate susceptibility falling into both groups (Tables 32 and 34). Overall, Bastile, a 6-row feed variety, was the most susceptible (Table 32). Out of the malting varieties, Conlon and Quest were the most susceptible, with severities of 42.5% and 35% in Tompkins-Ketola, respectively (Table 32). Although there were clear, statistically-supported differences between the most and least susceptible varieties overall,

none of the varieties had severities under 5% in Tompkins-Ketola in 2017 (Tables 32 and 34), indicating a lack of complete resistance to leaf rust.

Table 32. Mean leaf rust severities separated for SMBT varieties and the means of the two trials with the highest levels of leaf rust severity.

Variety		Overall Average (N = 14 plots per variety)	Percent severity at the trial with the highest level of leaf rust (Tompkins-Ketola 2017)	Percent severity at the trial with the second-highest level of leaf rust (Tompkins-Helfer 2016)
Bastile	a[x]	15.0	80.0	7.0
Harmony	ab	10.5	60.0	3.5
Conlon	ab	8.3	45.0	1.7
Quest	ab	5.6	30.0	0.5
AAC Synergy	ab	5.1	35.0	0
Oceanik	ab	4.3	25.0	0.3
Craft	ab	3.8	17.5	1
Newdale	b	3.7	25.0	0
Cerveza	b	3	20.0	0
Pinnacle	ab	2.5	15.0	1.2
KWS Tinka	b	2.1	12.5	0.3
ND Genesis	b	1.2	7.5	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 33. Significance of main effects and the interaction term from the ANOVA run on the transformed leaf rust severities of the SMBT varieties.

Effect	p-value	Level of significance[k]
Variety	0.002	**
Location	6.92E-11	***
Variety × Location	0.996	ns

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Table 34. Mean leaf rust severities separated for SMBT and ESBN varieties and the means of the two trials with the highest leaf rust severity.

Variety		Overall Average (N = 16 plots per variety)	Percent severity at the location with the highest level of leaf rust (Tompkins-Ketola 2017)	Percent severity at the trial with the second-highest level of leaf rust (Tompkins-Helfer 2017)
Conlon	a[x]	12.7	42.5	3.3
Quest	a	10.6	35.0	3.7
AAC Synergy	b	5.5	21.3	0.7
Newdale	b	5.5	21.3	0.5
KWS Tinka	b	3.2	11.5	0
ND Genesis	b	3.9	13.8	1.7
Pinnacle	b	2.7	9.5	0

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 35. Significance of main effects and the interaction term from the ANOVA run on the transformed leaf rust severities of the SMBT and ESBN varieties.

Effect	p-value	Level of significance[k]
Variety	3.63E-07	***
Location	< 2.2E-16	***
Variety × Location	0.0014	**

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Fusarium head blight

Ratable quantities of FHB were observed only in 2015, so a single analysis was done including the four locations of the SMBT from that year (Table 8). From the ANOVA, variety was found to be highly significant in explaining FHB index variability (Table 37). The varieties exhibiting the highest degree of FHB symptoms were ND Genesis, KWS Tinka, and Pinnacle, all of which had average FHB indexes over 1.5. The four varieties with the lowest FHB ratings were Cerveza, AAC Synergy, Quest, and Newdale.

Table 36. Mean FHB indexes separated for SMBT varieties and the means of the two trials with the highest levels of FHB.

Variety		Overall Average (N = 12 plots per variety)	FHB Index at the trial with the most FHB (Genesee 2015)	FHB Index at the trial with the second-highest level of FHB (Tompkins-Snyder 2015)
ND Genesis	a[x]	2.1	3.1	1
KWS Tinka	ab	1.7	2.3	3.6
Pinnacle	abc	1.6	4.3	1
Craft	bcd	0.8	1.9	0.2
Harmony	bcd	0.8	1.5	0.8
Conlon	bcd	0.6	0.1	0.7
Bastile	cd	0.6	1.3	0.7
Oceanik	cd	0.4	0.6	0.5
Cerveza	d	0.4	0.7	0.5
AAC Synergy	d	0.2	0.5	0.5
Quest	d	0.2	0.2	0.2
Newdale	d	0.2	0.4	0.1

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

Table 37. Significance of main effects and the interaction term from the ANOVA run on the transformed FHB indexes of the SMBT varieties.

Effect	p-value	Level of significance[k]
Variety	5.38E-11	***
Location	2.71E-06	***
Variety × Location	0.002	**

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Discussion

Based on its overall ubiquity and severity, it was determined that scald is the foliar disease that poses the greatest threat to winter malting barley in New York. Powdery mildew and leaf rust are both secondary threats to winter barley grown in the state. On spring barley, spot blotch, leaf rust, and powdery mildew all have demonstrated the ability to reach devastating levels on susceptible varieties when the environmental conditions favor disease. There are fewer varieties that appear to be resistant to spot blotch and leaf rust than for resistance to powdery mildew. For both winter and spring barley, Fusarium head blight develops in most years, and can infect all varieties.

The summaries of cultivar response to disease are in Table 38 for winter barley and in Table 39 for spring barley. The rankings that had been determined through statistical analysis were translated into a simpler rating score ranging from susceptible (S) to resistant (R), with the intermediate ratings of moderately susceptible (MS) and moderately resistant (MR). These tables are designed as an informational tool for non-scientists, especially farmers, to be able to easily compare varieties.

Table 38. Summary of winter barley cultivar disease resistance

Variety	Rows	Type	Variety's Developer	Scald	Leaf Rust	Powdery Mildew	Fusarium Head Blight
Charles	2	Malting	USDA-ARS, Aberdeen, ID	MS	S	R	MS
Endeavor	2	Malting	USDA-ARS, Aberdeen, ID	R	R	MR/R	MR
Flavia	2	Malting	Ackermann Saatzucht, Germany	S	R	R	S
KWS Scala	2	Malting	KWS, Europe	S	R	R	MS
Nectaria	2	Malting	Secobra, France	R	R	S	MS
Saturn	6	Feed	Limagrain, Europe	R	MR	R	MS
SY Tepee	2	Malting	Syngenta	R	R	R	MS
Wintmalt	2	Malting	KWS, Europe	S/MS*	R*	NA	MS

S = Susceptible, MS = Moderately Susceptible, MR = Moderately Resistant, R = Resistant

*Category was determined through observation because there was not enough power in the statistical test

Table 39. Summary of spring barley cultivar disease resistance

Varieties	Rows	Type	Variety's Developer	Spot Blotch	Leaf Rust	Powdery Mildew	Fusarium Head Blight
AAC Synergy	2	Malting	Agriculture and Agri-Food Canada	R	MS	R	R
Bastile	6	Feed	Synagri, Canada	R	S	R	MR
Cerveza	2	Malting	Agriculture and Agri-Food Canada	MR	MR	R	R
Conlon	2	Malting	North Dakota State University	MR	MS	R	MS
Craft	2	Malting	Montana State University	MR	MR	MS	MS
Harmony	6	Feed	Synagri, Canada	R	MS	R	MR
KWS Tinka	2	Malting	KWS, Europe	S	MR	R	S
ND Genesis	2	Malting	North Dakota State University	MS	MR	R	S
Newdale	2	Malting	Agriculture and Agri-Food Canada	MR	MR	R	R
Oceanik	6	Feed	Synagri, Canada	R	MS	R	MR
Pinnacle	2	Malting	North Dakota State University	S	MR	R	S
Quest	6	Malting	University of Minnesota	R	MS	S	R

S = Susceptible, MS = Moderately Susceptible, MR = Moderately Resistant, R = Resistant

There are a few caveats that must serve as an addendum to this analysis. The first is that this research was done in a limited number of locations in the state, and disease levels varied from year-to-year and from location-to-location, even within the same town. It is possible that some of these diseases, such as powdery mildew or leaf rust on winter varieties, could be more severe in microclimates not yet tested, or in years where weather patterns deviate from what was observed. Also, pathogen populations can shift to be more virulent. It is therefore possible for the observed genetic resistance to break down in subsequent years.

Finally, it has been observed that the visible symptoms of FHB on barley do not always correlate well with the amount of mycotoxin produced (Hill et al. 2007; de la Pena et al. 1999). The rankings determined for FHB in this report only pertain to the plant's visible response to the disease, and should be taken as a loose guideline rather than a guarantee that a given variety will remain disease- and mycotoxin-free, especially in years with weather highly-conducive to development of the disease.

These references may serve as a guide for growers, but disease resistance is not the only characteristic of the barley they need to consider before choosing a variety. They must also consider the agronomic and malting qualities of the cultivars. For example, AAC Synergy has demonstrated high resistance to spot blotch in the field, and it also has the highest yield, averaged over 2016 and 2017, out of the malting barley varieties in this study (Sorrells et al. 2017). One of its drawbacks is that it is prone to preharvest sprouting, where the seed begins to germinate before it has been harvested, thereby decreasing its malting quality. If a grower is looking for a high-yielding variety with lower pre-harvest sprouting they might select KWS Tinka (Sorrells et al. 2017), despite its high susceptibility to spot blotch. Growers must weigh

several factors when selecting a cultivar to plant, and disease resistance is only one element in that decision.

Also, in years where the risk for FHB is high, growers are likely to spray triazole fungicides at heading. This spray timing has been demonstrated to provide some control over the biotrophic fungi causing leaf rust and powdery mildew (Cummings et al. 2016a; Cummings et al. 2017), so genetic resistance to these diseases may not be something a grower looks for in the cultivar he or she chooses to grow. Alternatively, the hemibiotrophic leaf blights have not been controlled well by sprays at heading in spring barley (Cummings et al. 2017b). Therefore, disease resistance to spot blotch may be of economic value to a grower, who would have to consider an additional, earlier spray to manage the disease in a susceptible cultivar. Organic growers may find the disease susceptibility summary tables particularly useful because they do not yet have biological or chemical control options that have proved effective at controlling any of these diseases in field trials (Cummings et al. 2016b). Disease resistant cultivars are therefore their best option for disease management.

CHAPTER 3

DEVELOPING THE METHODS FOR GREENHOUSE EVALUATION OF SPOT BLOTCH RESISTANCE IN BARLEY

Introduction

Spot blotch, a disease caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*), has been identified as a potential threat to spring barley grown in New York State (Chapter 2). Without methods to study the pathogen experimentally, only observational studies relying on natural inoculum in the field can be performed. Observational studies are limited as to the scope of tests that can be performed and the conclusions that can be drawn from the results. Alternatively, experimental studies can be controlled, and can therefore be used to test specific questions posed by the researcher. Also, being able to perform experiments in the greenhouse rather than in the field allows researchers to decrease the size of their experiments, and allows them to perform their experiments year-round. The goal of this series of studies was to establish and test experimental methods that could broadly be used to test spot blotch in the facilities available at Cornell University, using isolates of the pathogen obtained in New York.

The overarching objectives were to:

1. Identify an aggressive New York isolate of *B. sorokiniana,* and determine what concentration of spores to use in greenhouse spot blotch screens
2. Choose susceptible and resistant check varieties of seedlings and adults of common two-row spring malting barley
3. Compare existing and novel methods of producing *B. sorokiniana* inoculum

Objective 1: Find an aggressive New York isolate of *B. sorokiniana*, and determine what concentration of spores to use in greenhouse spot blotch screens.

Methods

Obtaining single-spored isolates of B. sorokiniana *from diverse locations in New York*

In the summer of 2015, leaves exhibiting spot blotch symptoms were collected from spring malting barley in several locations around New York State. The leaves were surface-sterilized by soaking them for 30 seconds each in 90% ethanol, 10% bleach, and sterile water. Surface-sterilized leaves were placed onto PDA++ (39 g BD Difco™ Potato Dextrose Media, 0.12 g neomycin, 0.1 g streptomycin, 1 L DI H_2O) and the plates were placed under white light (34W, 12-hour photoperiod) to allow fungi to grow out from within the leaves. After four days, the most common fungus from each leaf was excised from the plate and the agar plug was placed onto a new PDA++ plate, which was then placed under black light (40W, 350nm peak wavelength, 12-hour photoperiod).

The fungal colonies were then single-spored to obtain isolates. After ten days of growth under black light, the plates were flooded with sterile, deionized water and scraped to release the spores. Thirty microliters of the spore suspension was plated onto a 1.5% water agar plate, put under white light, and left overnight. The next morning, germinating spores on the water agar were identified, cut from the plate, and placed on potato dextrose agar (PDA) (39 g BD Difco™ Potato Dextrose Media, 1 L DI H_2O). These plates were again placed under black light. Ten days later, conidia from the isolates were visually identified as *B. sorokiniana* and the spores were stored in 15% glycerol stock at -80ºC.

Selecting isolates for greenhouse spot blotch screens

Four of the isolates collected in 2015 were selected to trial in greenhouse spot blotch screens (Table 40). These isolates were selected for the geographic diversity of where they were collected.

Table 40. Description of isolates chosen for greenhouse screening

Isolate	Town where isolate was collected	County where isolate was collected	Barley variety
Bs197NY15	Ithaca, NY	Tompkins	Unknown
Bs225NY15	Wayland, NY	Steuben	Unknown
Bs228NY15	Aurora, NY	Cayuga	Conlon
Bs233NY15	Franklin, NY	Delaware	Conlon

The species of the chosen isolates was verified by sequencing their ITS1 and ITS2 regions. The isolates were taken from their -80ºC glycerol stocks, grown for five days on PDA and then transferred onto new PDA plates. These PDA plates were sealed with parafilm, placed in a dark incubator set to 21ºC for 10 days to allow the *B. sorokiniana* to grow (Arabi and Jawhar 2013), and then stored at 2ºC until the mycelium could be collected for DNA extraction.

The mycelium of each of the isolates was scraped from the surface of its stock plate, and disrupted with garnet beads by shaking it in bead-beating tubes. DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and stored at -20ºC.

PCR was run using the universal primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). The reaction mixture was 5.5 µl H_2O, 12.5 µl GoTaq® (Promega, Madison, WI), 1 µl of each primer, and 5 µl of the DNA. The program used was 95ºC for 10 min; 30 cycles of 95ºC for 1 min, 55ºC for 1 min, 72ºC for 90s; and then 72ºC for 10 min (Bio-Rad MyCycler Thermal Cycler, Hercules, CA). The ITS PCR products were sequenced in both directions at Cornell University's Biotechnology Resource

Center (Applied Biosystems® 3730xl DNA Analyzer, Foster City, CA). NCBI BLAST revealed that the isolates had 99% identity matches to *B. sorokiniana*.

Obtaining a dose-response curve for each of the four isolates on a susceptible barley cultivar

Experimental design

The four isolates of *B. sorokiniana* were tested at five inoculum concentrations to determine what concentration of spores would cause 50% tissue death on the susceptible two-row spring barley cultivar, Full Pint. The five inoculum concentrations were 10, 10^2, 10^3, 10^4, and 10^5 conidia/mL. The experimental layout was a randomized complete block design with the replicates blocked. Each block contained a completely randomized set of all the treatments (4 isolates x 5 spore concentrations) and one control that was treated with a mixture of water and 1% TWEEN20 (Polysorbate 20), for a total of 21 different treatments. The experiment was run twice.

Growing plants

Full Pint was planted in 4-inch pots, four seeds per pot, in Cornell Mix (3.8 bales peat moss, 9.1 kg vermiculite, 9.1 kg perlite, 2.3 kg lime, 1.8 kg Jack's Professional 10-5-10 Media Mix Plus III (JR Peters Inc, Allentown, PA), 0.11 kg AquaGro® wetting agent (Aquatrols, Paulsboro, NJ)). The plants were grown in the greenhouse (22ºC, 12-hour light cycle, watered once daily with tap water (0.34 mg/L chlorine) with 300 ppm nitrogen added twice weekly). Before inoculation, excess seedlings were removed so that there were only two plants per pot. Plants were grown for 15 days, to the second leaf stage.

Growing isolates

Two days after the barley was planted, plug transfers were made from the stock plates made directly from the glycerol stocks stored at -80ºC. The plugs were placed onto fresh PDA plates, sealed with parafilm, and put into a dark incubator set to 21ºC (Arabi and Jawhar 2013).

Inoculum preparation

Fifteen days after the barley was planted and thirteen days after the isolates were plated, the inoculum was prepared. Each culture plate was flooded with 10 mL of water and its surface was scraped with a bent glass rod. Spores for each isolate was collected separately.

The resulting spore suspensions were filtered through two layers of cheesecloth to remove mycelium. The spore concentration for each isolate was quantified using a hemocytometer: two measurements were made for each spore suspension and averaged. A serial dilution of 10, 10^2, 10^3, 10^4, and 10^5 cells/mL of each isolate's spore suspension was made. In the first repetition of the experiment, Bs225NY15 had a maximum concentration of 91,000 spores/mL. In the second repetition, only10^4 spores/mL of Bs228NY15 and Bs233NY15 and only 9.04 x 10^4 spores/mL of Bs225NY15 were obtained. The surfactant, TWEEN20 was added to each spore suspension (100ul/L) to break surface tension and increase surface coverage. A control solution of water and TWEEN20 (100ul/L) was also made.

Plant inoculations

The second leaf of each plant was sprayed to achieve maximal coverage of the leaf surface with fine droplets without causing coalescence of the droplets or runoff of suspension using an aerosol-based sprayer (Preval® Sprayer, Chicago Aerosol, Coal City, IL). To allow the spores to reach the leaf surface, the leaves were allowed to dry before the pots were maneuvered. Once the leaves had dried, the plants were enclosed in 13-gallon white plastic bags, which served

as moist chambers. The bags were sealed after the plants were heavily misted with water (Fig. 1). Seventeen hours after being enclosed, the bags were reopened, the plants were allowed to dry, and the pots were moved back into random order.

Figure 1. Seven pots were set at the bottom of each 13-gallon white plastic bag, serving as a moist chamber, and were left sealed overnight.

Rating

Ten days post-inoculation (dpi), ratings were taken as a visual estimate of the percent leaf area covered by spot blotch lesions on the second leaf. The severities of the two plants in each pot were averaged.

Analysis

The two trials were analyzed separately. One outlier from isolate Bs233NY15 applied at 10^3 spores/mL was removed from the first trial because its severity was more than two standard deviations greater than the mean of the group. The severities for each treatment were averaged, and the logarithm was taken of those averages. A dose-response curve (log10(severity) vs. log10(concentration)) was created for each isolate. The linear portion of each curve was selected, and a linear regression was calculated. From the resulting linear equations, the inoculum concentration at which the leaves would be 50% covered by lesions was estimated for each

isolate in each trial. The two concentrations calculated for each isolate were averaged, and the most aggressive isolate was selected for future analyses.

Results

In general, disease developed better in the first trial than it did in the second. In the first trial, the maximum severity observed was an average of 83.6% disease on plants treated with isolate Bs225NY15 at a concentration of 10^5 conidia/mL. In the second trial, the maximum disease severity observed was an average of 41.4% disease on plants treated with isolate Bs197NY15 at a concentration of 10^5 conidia/mL. The lower disease severity in the second trial

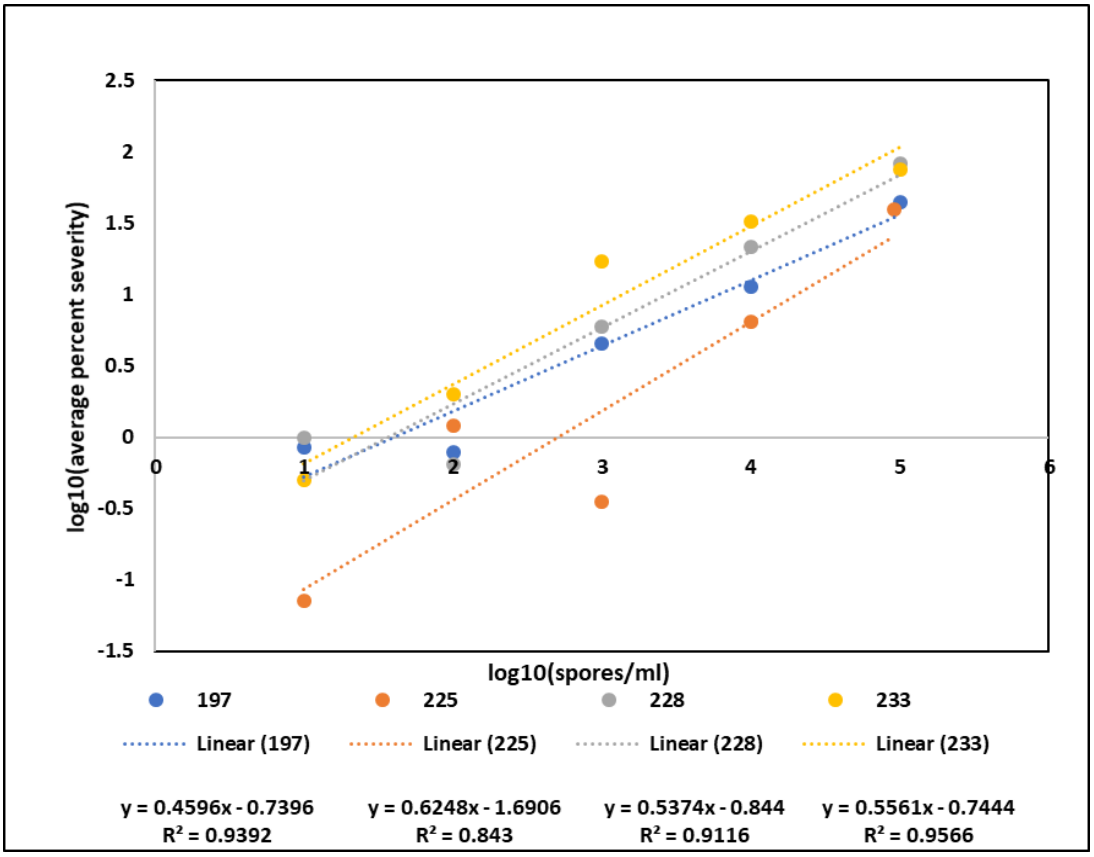

Figure 2. Log-log dose-response curve for the isolates Bs197NY15 (blue), Bs225NY15 (orange), Bs228NY15 (gray), and Bs233NY15 (yellow) applied on Full Pint, from the first trial. The equation of each isolate's regression line and the corresponding R^2 value is reported below the graph.

resulted in more treatments that resembled the nontreated controls, meaning that the linear portion of the log-log dose response curve was smaller for the second trial, fewer points were included in each curve, and support for each regression was less robust (Fig. 2 and Fig. 3). The R^2 for all regressions were no lower than 0.8 (Fig. 2 and Fig. 3).

Regardless of the trial, isolate Bs233NY15's calculated inoculum concentration required to produce symptoms on 50% of leaf tissue was the lowest, with an average of 3.2 x 10^4 conidia/mL (Table 41). Isolate Bs197NY15 had the next lowest average, requiring 2.2 x 10^5 conidia/mL, and Bs225NY15 required 3.8 x 10^5. Isolate Bs228NY was the only isolate that had a

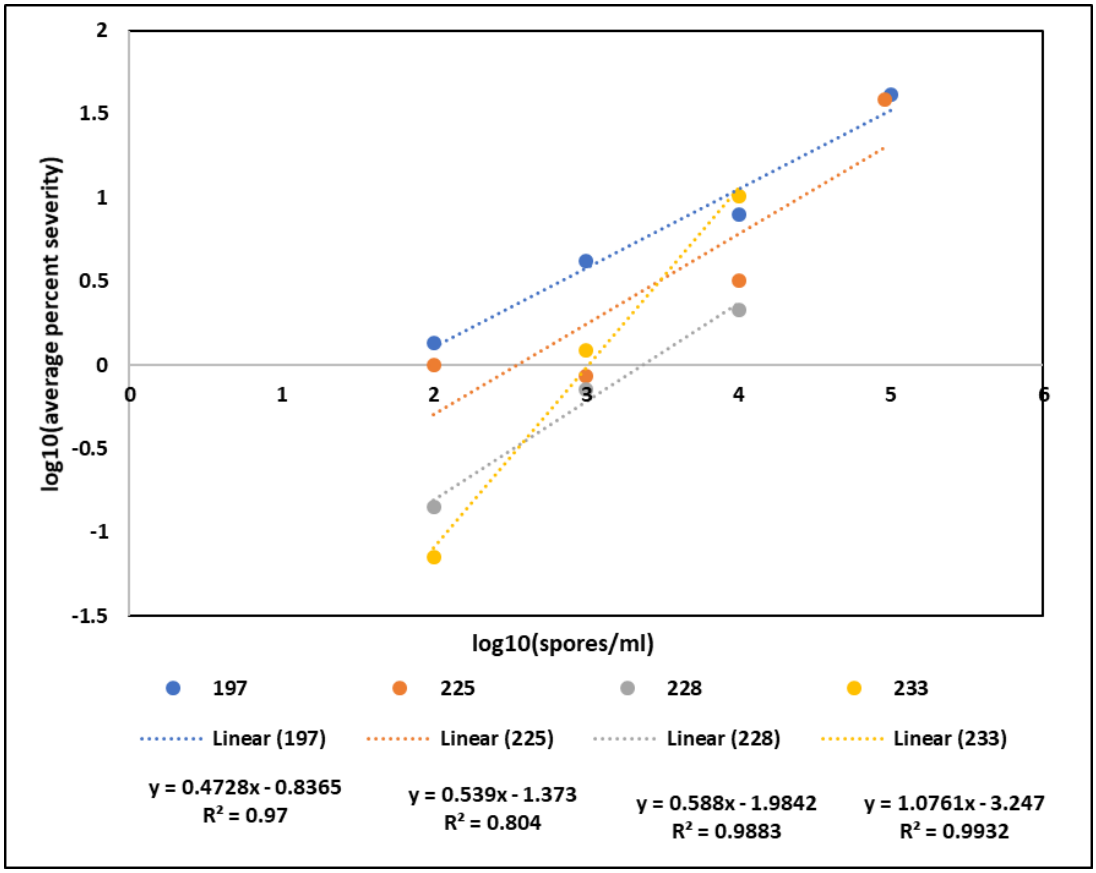

Figure 3. Log-log dose-response curve for the isolates Bs197NY15 (blue), Bs225NY15 (orange), Bs228NY15 (gray), and Bs233NY15 (yellow) applied on Full Pint, from the second trial. The equation of each isolate's regression line and the corresponding R^2 value is reported below the graph.

large disparity in ability to cause disease between the two trials. In the second trial, it was calculated that inoculum would need to be 33 times stronger to have the same potency as in the first (Table 41).

Table 41. The concentration of inoculum required to cause symptoms on 50% leaf tissue on Full Pint seedlings 10 days post-inoculation, calculated for each trial independently, and as an average of the two trials.

	Inoculum concentration (spores/mL) to reach 50% disease severity		
Isolate	**Trial 1**	**Trial 2**	**Average**
Bs197NY15	2.0×10^5	2.3×10^5	2.2×10^5
Bs225NY15	2.7×10^5	5.0×10^5	3.8×10^5
Bs228NY15	5.4×10^4	1.8×10^6	9.4×10^5
Bs233NY15	2.5×10^4	3.9×10^4	3.2×10^4

Conclusions

Bs233NY15 was consistently the most aggressive isolate, and it was therefore chosen as the isolate to be used for all subsequent greenhouse screens. The concentration at which Bs233NY15 kills 50% leaf tissue on the susceptible cultivar, Full Pint, was found to be 3.2×10^4 conidia/mL. Isolate Bs228NY15 was chosen to use in some future screens as well because, despite its low aggressiveness in the second trial, its performance in the first trial was an order of magnitude better than the other two isolates. It also has a better-recorded provenance than either Bs225NY15 or Bs228NY15 because it is known to have come from the cultivar, Conlon, which is the same cultivar Bs233NY15 was isolated from.

Objective 2. Choose susceptible and resistant check varieties of two-row spring malting barley

When doing disease screens, it is useful to include resistant and susceptible check varieties to verify that the assay worked and to serve as points of comparison for the rest of the screened varieties. The main goal of this set of experiments was to choose check varieties for future seedling and adult spring 2-row barley spot blotch screens using *B. sorokiniana* isolate Bs233NY15. Additional goals included 1) ranking the seedling and adult spot blotch susceptibility of the parents used in Cornell University's two-row spring barley breeding program: AAC Synergy, Bentley, Conlon, Craft, ND Genesis, Newdale, and KWS Tinka and 2) testing for genotype × isolate interactions.

Two different experiments were run to complete this objective. The first was a spot blotch evaluation on seedlings. The second was a nearly-identical screen on adults. The two separate screens were conducted because it has been well-documented that disease resistance can differ between seedling and adult plants of the same genotypes (Parlevliet 1979), and there is evidence that the genes playing a role in barley response to spot blotch can differ between these two growth stages (Steffenson et al. 1996; Bilgic et al. 2005; Bovill et al. 2010).

Methods

Experimental design

A randomized complete block design was used to test both the seedling and adult plant resistances to spot blotch. There were two factors tested: cultivar and isolate. The 12 cultivars tested were the two-row spring barley varieties AAC Synergy, AC Metcalfe, Bentley, Conlon, Craft, Full Pint, Hockett, KWS Tinka, ND Genesis, Newdale, Pinnacle, Scarlett. The three isolate treatments were isolate Bs225NY15, isolate Bs233NY15, and no inoculum using a water

control. Within each replicate, a complete factorial of possible treatment combinations, a total of 36 treatments, were completely randomized. Five replicates were run at the same time for the seedling trials, and four replicates were run at the same for the adult trials. Three full repetitions of the experiment were run for both the seedling and the adult screens.

Plant material

Each cultivar was planted three seeds to a pot using Cornell Potting Mix. The seeds for the seedling experiments were planted in 4-inch pots, and the seeds for the adult screen were planted in 6-inch pots. The plants were grown in the greenhouse (22ºC, 12-hour light cycle, watered once daily). For the seedling trials, excess seedlings were removed so that there were only two plants per pot at least one day before inoculation. Seedlings were inoculated 15 days after planting, at approximately the second leaf stage.

For the adult screens, plants were removed at about the four-leaf stage, so that there was only one plant left per pot. At least a day before inoculation, all but three tillers at the heading growth stage were removed. Adult plants were inoculated at heading, approximately 70 days after planting.

Bipolaris sorokiniana production

Stock plates were made by plating isolates Bs225NY15 and Bs233NY15 from the glycerol stocks stored at -80ºC directly onto V8 agar (163 mL V8 juice, 2.44 g $CaCO_3$, 12.2 g agar, 650 mL DI H_2O). After about 10 days of growth under black light (40W, 350 nm peak wakelength), these plates were used to make a highly-concentrated spore suspension. New plates for each isolate were created by pipetting 75 µl of the spore suspension onto each V8 plate and spreading the suspension using a sterilized L-shaped glass rod. The plates were grown under

black light (12-hour photoperiod) for ten days before the spores were collected for use in inoculum.

Inoculum production

After the isolates had grown for 10 days, the spores were collected by flooding each plate with 10 mL water and gently scraping the surface of the agar with an L-shaped glass rod. The spore suspension was collected in a separate beaker for each isolate. The spore suspensions were filtered through three layers of cheesecloth. Spores were counted using a hemocytometer, and the concentration of the spore suspension for each isolate was adjusted to 3.2×10^4 spores/mL for the seedling screens and to 4.0×10^4 spores/mL for the adult screens. The surfactant, TWEEN20 was added to the spore suspensions (100ul/L). The control solution was made using H_2O and TWEEN20 (100ul/L).

Inoculation

Seedlings were inoculated when the second leaf was fully emerged, and only the second leaf was inoculated. The adults were inoculated at the heading growth stage, and the flag leaf and the second leaf from the top were both inoculated. The four replicates included in each adult trial had to be inoculated one day apart to be able to fit all the plants in the mist chamber.

Inoculations were performed by spraying the leaves with a fine mist of inoculum to achieve maximal coverage of the leaf surface with fine droplets without causing coalescence of the droplets or runoff of suspension using Preval® aerosol sprayers. The plants were all allowed to dry before being moved again. Once the seedlings' leaves were dry, the plants were enclosed in 13-gallon white plastic bags, which served as moist chambers. The bags were sealed after the plants were heavily misted with water. Seventeen hours after the bags were closed, they were

reopened, and the plants were allowed to dry before the pots were moved back into random order.

Once the adults' leaves were dry, the pots were moved into the mist chamber (set to 22ºC, 90% humidity, with no additional lighting). After the plants had been in the mist chamber for 17 hours, the mist chamber was switched off and the doors were opened to allow the plants to dry. Once the plants had dried, the pots were moved back into the greenhouse.

Rating

Ten days post-inoculation, ratings were taken as a visual estimate of the percent leaf area covered by lesions on the leaves that had been sprayed with inoculum. This included the second leaf for the seedlings, and the top two leaves for the adults. The spot blotch severity on the two seedlings in each pot were averaged, and the severity on the three tillers of each adult plant were averaged, resulting in a single severity score per pot.

Analysis

Spot blotch severity data for seedlings and adults were analyzed using analysis of variance (ANOVA). The non-treated controls were not included in the analysis, as they all had zero or close to zero disease severity. The effects included in the seedling ANOVA were variety, isolate, trial date, and replicate within trial, and the interactions included were variety × isolate, variety × trial, isolate × trial, variety × isolate × trial, and isolate × replicate. The effects included in the adult ANOVA were variety, trial, isolate, and replicate within trial. The only interaction term included was isolate × replicate. Means were separated using Tukey's HSD ($\alpha = 0.05$). The Pearson correlation was calculated between average spot blotch severity on seedlings and adults.

Results

Seedling spot blotch screen

There was a broad range of susceptibility to *B. sorokiniana* found in the 12 cultivars tested. Craft had the highest severity observed, reaching 69% severity when inoculated with isolate Bs233NY15 (Fig. 4). The most susceptible cultivar overall was Full Pint, with an average spot blotch severity of 44.2% when both isolates were taken into account (Table 42). These two varieties were also found not to be statistically significantly different from Scarlett (Table 42). Newdale had the lowest spot blotch severity, at 12.5%, but AAC Synergy, which had an average severity of 13%, was very similar. Several cultivars were found to be statistically indistinguishable from the least susceptible varieties. The cultivars that grouped into the least susceptible group include Newdale, AAC Synergy, ND Genesis, Bentley, Pinnacle, and KWS Tinka.

The variety × isolate interaction was found to be highly significant from the ANOVA ($p < 0.001$) indicating that the ranking of the susceptibility of a cultivar in the screen was dependent on which isolate it was inoculated with. This was particularly true for the varieties that were appraised as the most susceptible overall to spot blotch. Craft had a 55% difference in severity between plants inoculated with isolate Bs228NY15 and those inoculated with Bs233NY15 (Fig. 4). Scarlett and Full Pint were the only other two varieties with statistically-distinguishable severity differences between isolates.

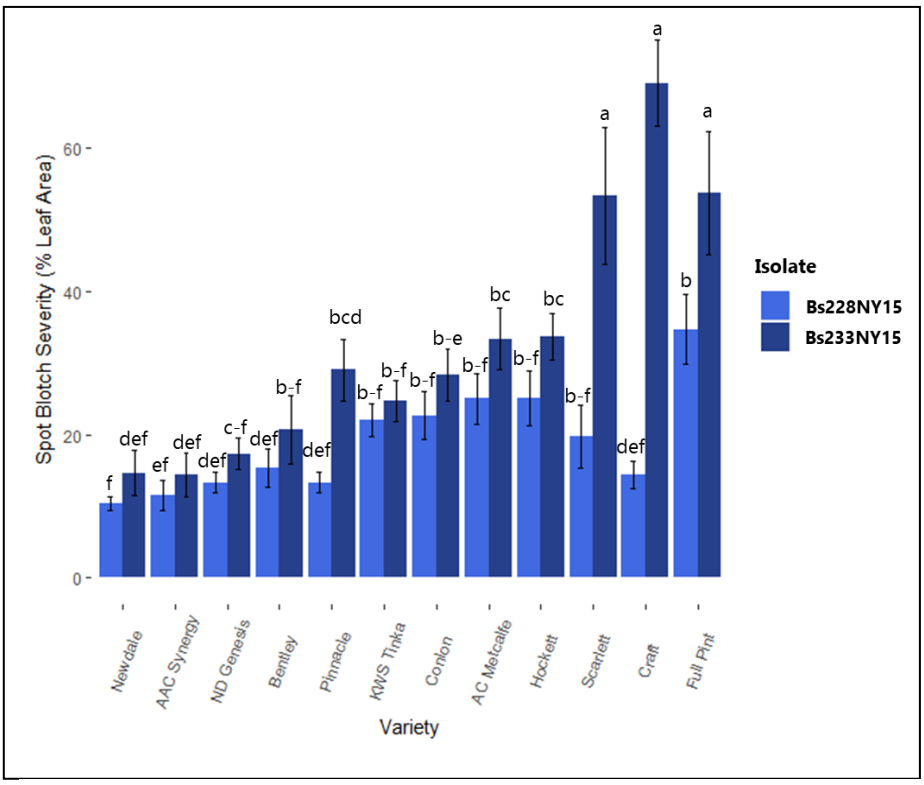

Figure 4. Average spot blotch severity on seedling two-row spring barley cultivars inoculated at the two-leaf growth stage with either *B. sorokiniana* isolate Bs228NY15 or Bs233NY15. Error bars represent a single standard error above and below the mean. Groups labeled with the same letter do not statistically differ, $P = 0.05$

Table 42. Mean spot blotch severity on two-row spring barley cultivars, screened as seedlings.

Variety		Mean Spot Blotch Severity (%)
Full Pint	a[x]	44.2
Craft	a	41.7
Scarlett	ab	36.5
Hockett	bc	29.4
AC Metcalfe	bc	29.2
Conlon	cd	25.5
KWS Tinka	cde	23.3
Pinnacle	cde	21.2
Bentley	de	18.0
ND Genesis	de	15.3
AAC Synergy	e	13.0
Newdale	e	12.5

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$

A variety of lesion types were observed on the seedlings 10 dpi. The most common symptom types fell into one of three categories. The more susceptible varieties, like Full Pint and Scarlett, tended to have dark centers, delimited by the veins on the leaf, so that the spots appeared round or oval. The dark centers were surrounded by bright yellow, chlorotic halos (Fig. 5, B & C). The more resistant varieties, such as ND Genesis, tended to have smaller lesions with necrotic centers and dark halos (Fig. 5, D). The varieties that had moderate susceptibility to spot blotch, like Conlon, had intermediate symptoms. The lesions on these cultivars started small, but began to spread down the leaf in a long band, largely without crossing over the leaf veins. These lesions were occasionally surrounded by a chlorotic halo (Fig. 5, A), and overall resemble the description for the barley disease, net blotch, caused by *Pyronophora teres*.

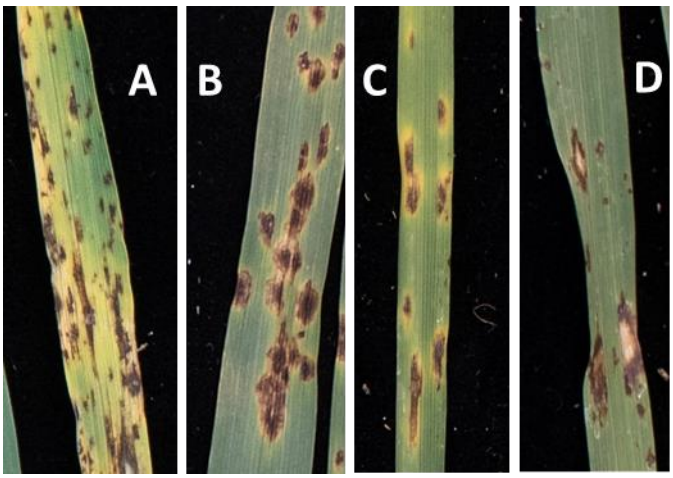

Figure 5. Spot blotch symptoms caused by isolate Bs225NY15 on Conlon (A), Full Pint (B), Scarlett (C), and ND Genesis (D). The leaf sections displayed were chosen for symptom development representative of the cultivar, rather than representative of the spot blotch severity observed on the cultivar.

Craft was the only variety that had a different lesion type when inoculated with different isolates. Plants inoculated with Bs228NY15, developed lesions with a necrotic center surrounded by a dark halo (Fig. 6), similar to the lesions seen on the more resistant varieties like on ND Genesis (Fig. 5, D), AAC Synergy, and Newdale. With Bs233NY15, the lesions appeared to be dark at the center with a bright tan or yellow halo surrounding each lesion (Fig. 6). This was more similar in appearance to the susceptible varieties Full Pint and Scarlett (Fig. 5, B & C).

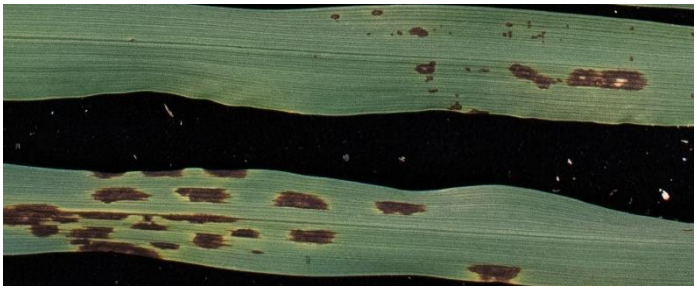

Figure 6. Symptoms of spot blotch on Craft. The leaf on the top was inoculated with Bs225NY15, and the leaf on the bottom was inoculated with Bs233NY15. Craft was the only variety for which the lesions looked dramatically different between the two isolates.

Adult spot blotch screen

Isolate was not found to be significant in the adult screen, so the data for the two isolates are reported together (Fig. 7). The range in spot blotch severity from the most to the least susceptible cultivar at the adult stage was 28% (Figure. 7), which is similar to the 32% range found for the seedlings (Table 42). Scarlett, had the highest average spot blotch severity, and AAC Synergy and Bentley had the lowest.

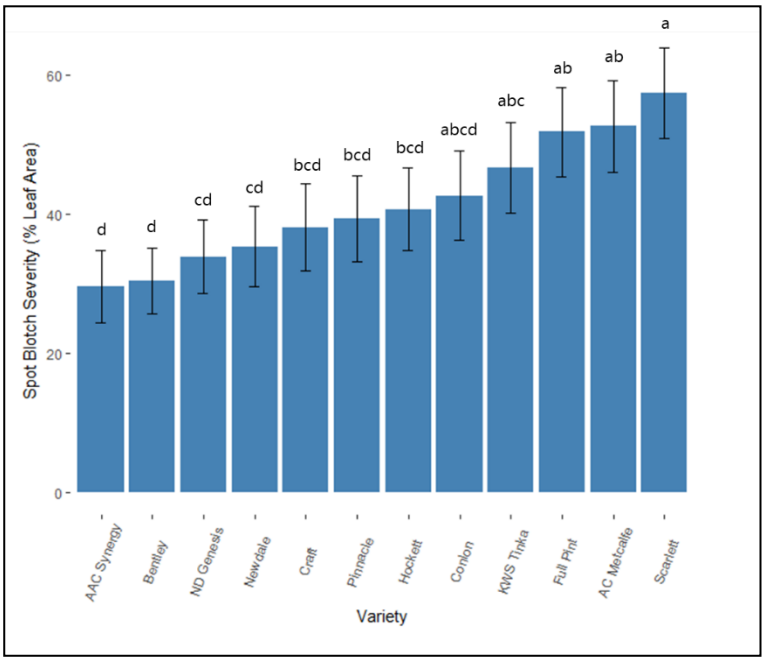

Figure 7. Average spot blotch severity on adult two-row spring barley cultivars inoculated at the heading stage. Error bars represent a single standard error above and below the mean. Groups labeled with the same letter do not statistically differ, $P = 0.05$

Comparison between seedling and adult spot blotch susceptibility

There was a strong positive correlation found between seeding and adult susceptibility to spot blotch (Fig. 8). Craft was the variety that changed most drastically in rank between seedling and adult resistance (Fig. 9)

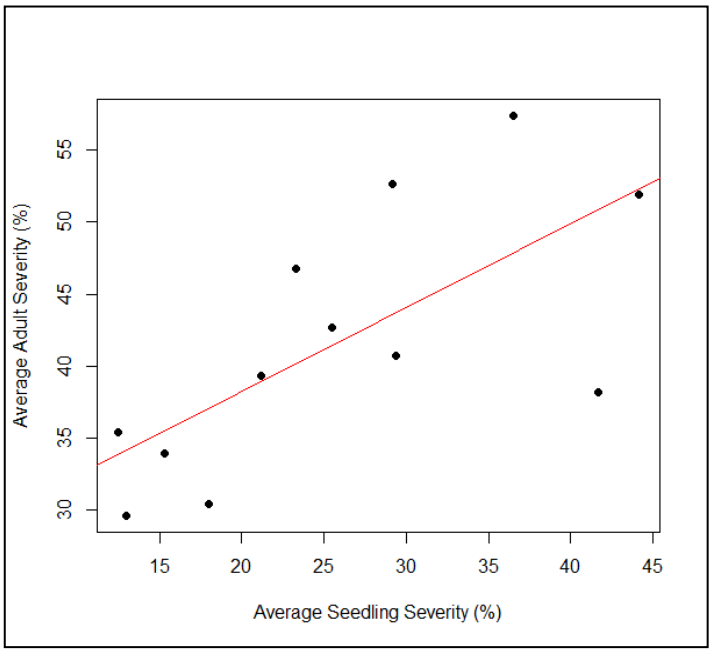

Figure 8. Correlation between average spot blotch severity on seedlings and adults of 12 barley cultivars. Pearson's correlation coefficient = 0.696, *p*-value < 0.001.

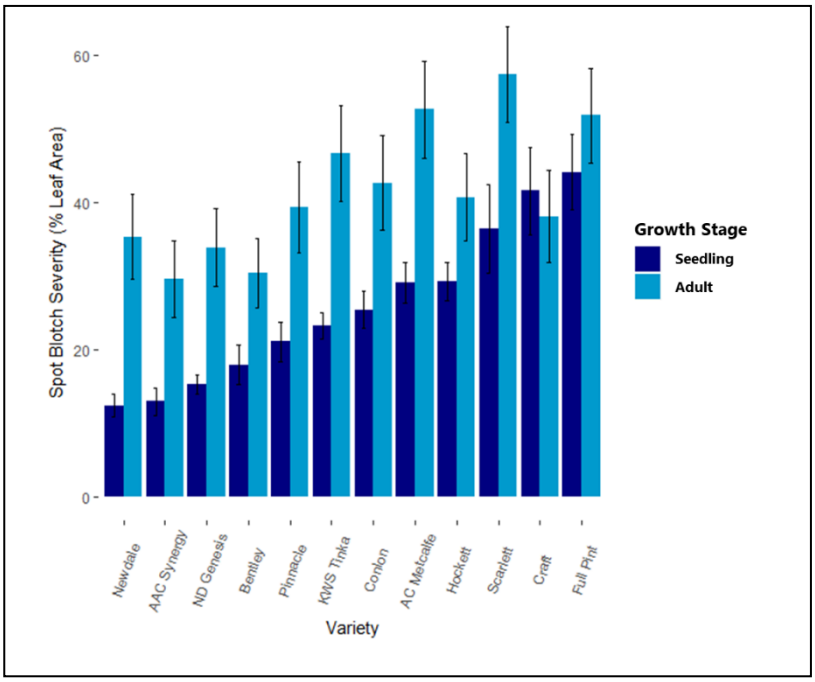

Figure 9. Comparison of the average spot blotch severity on adult and seedling two-row spring barley cultivars. Error bars represent a single standard error above and below the mean.

Conclusions

Overall, it was found that the twelve two-row spring barley cultivars tested have a range of susceptibility to spot blotch, at both seedling and adult growth stages. Full Pint was the most susceptible cultivar at the seedling stage, while Scarlett was the most susceptible cultivar at the adult stage. Newdale was the most resistant cultivar at the seedling stage and AAC Synergy was the most resistant at the adult stage. These are therefore the best candidates for use as susceptible and resistant checks in future spot blotch screens.

Additionally, the significant variety × isolate interaction for seedlings provides evidence that it could be worthwhile to determine what pathotypes or races of *B. sorokiniana* exist in New York. Since only two isolates were screened on all twelve varieties, it may be that there are other pathotypes that are present in the state that are more virulent on some cultivars than was observed in the greenhouse. Some evidence for this hypothesis is that Pinnacle was found to have the highest adult plant susceptibility to spot blotch in the field during the 2015-17 field seasons (Chapter 2). The rankings in the field do not align well with those found in the greenhouse, where Pinnacle was only found to have moderate susceptibility to spot blotch. ND Genesis was also found to be comparatively more susceptible in the field (Chapter 2).

Objective 3. Compare existing and novel methods of producing *B. sorokiniana* conidia for use as inoculum

In our first attempts to grow *B. sorokiniana* for inoculum, difficulties were encountered in obtaining reliable quantities of spores. For example, in the test of the isolates on Full Pint (Objective 1), the methods from Arabi and Jawhar (2013) were replicated, but inadequate quantities of spores were collected in the second trial to test three of the isolates at the full 10^6 spores/mL concentration. One of the main problems encountered was that the cultures sectored:

parts of the culture would only produce mycelium, while other parts of the plate would produce dense mats of conidiophores and conidia. These methods were therefore deemed unreliable.

In the literature, there is no consensus on how *B. sorokiniana* should be grown for use in screening the isolates for pathogenicity or for use in screening cultivars for resistance. Some of the most informative articles published on screening barley seedlings for response to *B. sorokiniana*, have come from Dr. Brian Steffenson's labs at the University of Minnesota and North Dakota State University (Fetch and Steffenson 1999; Haas et al. 2016) and from Dr. Mohamad Arabi's lab at the Atomic Energy Commission of Syria (Arabi and Jawhar 2007, 2010, 2013). Dr. Steffenson's lab members often use the methods published in Fetch and Steffenson (1999), where *B. sorokiniana* is grown on Yeast Extract Corn Starch Agar (YECSA) under fluorescent light (12-hour photoperiod) for ten days. Dr. Arabi's lab uses the methods published in Arabi and Jawhar (2013). In this case, *B. sorokiniana* is grown on potato dextrose agar amended with antibiotics and grown in the dark for ten days. Other published methods for producing *B. sorokiniana* inoculum include using V8-PDA either in constantly light or constantly dark conditions (Maraite et al. 1997), 30% V8 agar under constant light (Duveiller and Garcia Altamirano 2000), minimal medium under 14-hour fluorescent light (Leng et al. 1988), and yeast peptone soluble starch media with a 12-hour photoperiod (Bilgic et al. 2006).

In this study, two distinct experiments were run to evaluate potential inoculum production techniques. The first was to test whether adding barley seed, one of *B. sorokiniana*'s natural substrates, to the media would increase spore production. This experiment was performed jointly with research support specialist Jaime Cummings. The second was to do a comparison of published (Fetch and Steffenson 1999; Arabi and Jawhar 2013) and unpublished methods by

testing different media types, light sources, and transfer methods, to identify an effective set of conditions to grow sporulating *B. sorokiniana* for future use in Dr. Bergstrom's lab.

Methods

Growing B. sorokiniana on V8 agar media amended with ground barley kernels

Experimental design

This experiment was done in a complete factorial design with two factors: media and transfer technique. The two levels of media type were plain V8 agar and V8 agar amended with ground barley. The two levels of transfer technique were plug-transfer and spore suspension-transfer. The 2 × 2 factorial design resulted in four treatment combinations, each of which was replicated five times.

The V8 agar (163 mL V8 juice, 2.44 g $CaCO_3$, 12.2 g agar, 650 mL DI H_2O) was poured into small, 5 cm-diameter petri dishes. The V8 barley agar (V8 agar + 50 g ground barley kernels) was poured in standard, 8.5 cm-diameter petri dishes.

Stock plates of *B. sorokiniana* isolate Bs233NY15 were made by spreading a small amount of the 15% glycerol stock stored at -80ºC, onto PDA plates and placing them under black light (40W, 350 nm peak wavelength,12-hour photoperiod) and allowing them to grow for several days. On five V8 agar and five V8 barley agar plates, the *B. sorokiniana* was transferred by cutting a small cube (2 mm) of agar from the edge of the fungal growth on the stock Bs233NY15 plate and placing it at the center of the new plate. This was the 'plug-transfer' method. On five V8 agar and five V8 barley agar plates a small volume (less than 1 mL) of a highly-concentrated spore suspension made from the stock Bs233NY15 plate was pipetted onto and spread thoroughly over the surface of the new plate using a bent glass rod. This was the

'spore suspension-transfer' method. The 20 prepared plates were placed under black light (12-hour photoperiod) to grow for eight days.

Spore quantification

After eight days, spore production was quantified for each plate. Five milliliters of water were used to flood the small plates and 10 mL of water was used to flood the 8.5 cm diameter plates. The surface of each plate was gently scraped with an L-shaped glass rod for 30 seconds to release the spores into the water. A small volume of well-mixed spore suspension was pipetted directly from the plate onto a hemocytometer. Two independent spore counts were taken for each plate, the average of which was used to calculate the concentration of spores. The spore production per cm^2 of surface area was calculated by dividing the total spore production for the plate by the surface area of the plate (19.6 cm^2 for the small plates and 56.7 cm^2 for the regular-sized plates).

Statistical analysis

The data were analyzed using ANOVA with media and transfer technique as main effects, and media × transfer technique as an interaction term.

Comparing media, light source, and transfer method in producing B. sorokiniana *inoculum*

Experimental design

This experiment was done in a complete factorial design with three factors: media type, light source, and transfer technique. The three levels of media type were potato dextrose agar amended with antibiotics (PDA++), yeast extract corn starch agar (YECSA), and V8 agar. The three levels of light source were black light (12-hour photoperiod), white light (12-hour photoperiod), and no light. The three levels of transfer technique were plug-transfer, spore suspension-transfer from a spore suspension of 40,000 spores/mL, and spore suspension-transfer

from a spore suspension of 80,000 spores/mL. The 3 × 3 × 3 factorial design resulted in 27 treatment combinations, each of which was replicated five times.

PDA++ (39 g BD Difco™ Potato Dextrose Media, 0.12g neomycin, 0.1g streptomycin, 1 L DI H_2O), YECSA (1.0g KH_2PO_4, 0.5 g $MgSO_4 \bullet H_2O$, 4.0 g BD Bacto™ yeast extract, 15 g corn starch, 20 g agar, 1 L H_2O), and V8 agar (163 mL V8 juice, 2.44 g $CaCO_3$, 12.2 g agar, 650 mL DI H_2O) were all poured into standard-sized, 8.5 cm-diameter petri dishes.

Stock plates of *B. sorokiniana* isolate Bs233NY15 were made by spreading a small amount of the 15% glycerol stock stored at -80ºC onto V8 agar. The plates were grown under black light (12-hour photoperiod) for 10 days. Once the stock plates had fully grown, the fungus was transferred onto 15 plates of each media type by cutting a small cube of agar from the most recent fungal growth and placing it at the center of a new plate. This was the 'plug-transfer' method. A spore suspension was made from the stock plates, and its concentration was adjusted so that there were two spore suspensions, one at a concentration of 40,000 spores/mL and the other at a concentration of 80,000 spores/mL. For 15 plates of each media type, 75µl of the 40,000 spores/mL solution was pipetted onto each and spread with an L-shaped glass rod. The same was done with the 80,000 spores/mL solution. Collectively, these were prepared using the 'spore suspension-transfer' method.

Five plates of each transfer-type × media treatment were placed under one of three different types of lighting conditions. One group was placed under fluorescent light (34W), another group was placed under black light (40W, 350 nm peak wavelength), and the third group was placed in the dark. Both light sources were set to a 12-hour photoperiod. The plates were left undisturbed for 10 days, after which data were collected.

Data collection

Spores were collected by flooding each plate with 10mL of H_2O and gently scraping the surface of the agar with a bent glass rod for 30 seconds. Using a volumetric pipette, as much of the suspension was removed from the plate as possible. The samples were stored in 10mL plastic test tubes until the spores could be counted and quantified. Three metrics were recorded for each plate: volume of water absorbed by the mycelium on the plate, total spore production, and average spore length. The water absorbed by mycelium was considered to be a proxy for the amount of mycelium produced. This was calculated because large amounts of mycelium production can be problematic while filtering the spore suspension to make inoculum. The conidial length was considered to be a metric of how well-developed the conidia were.

The volume of water absorbed by the mycelium was calculated by subtracting the volume of spore suspension obtained from the plate from the original 10 mL pipetted onto the plate. The total number of spores produced was estimated by counting spores using a hemocytometer, calculating the concentration, and multiplying the spore concentration by the volume of the spore suspension obtained from the plate. While using the hemocytometer, two independent counts were taken per sample and averaged. To obtain the average spore length, ten random spores per sample were measured at their maximum length, using the ocular reticle of the light microscope.

Statistical analysis

Total spore production, average spore length, and water absorbed by mycelium were each analyzed separately using ANOVA. Media, light source, and spreading techniques were analyzed as main effects, and all four possible interaction terms were included. The 40,000 spores/mL and 80,000 spores/mL spore suspension-transfers were not found to be statistically distinct in any

case, so they were combined into a single category, hereafter called spore suspension-transferred. Means were separated into significant groupings with Tukey's HSD ($\alpha = 0.05$).

Results

Growing B. sorokiniana *on V8 agar media amended with ground barley kernels*

The fungus exhibited different growth patterns for each of the different treatments (Fig. 10). The plug-transfer plates tended to lead to sectioning of the fungus, with some areas producing spores and others producing only mycelium (Fig. 10, A). The plates made with a spore suspension had more uniform growth, with random patches of mycelium emerging from a field of sporodochia (Fig. 10, C & D). The mycelium on the V8 barley agar plates grew more thickly and more compactly (Fig. 10, D), while the mycelial masses on the V8 agar plates were thinner and more thread-like (Fig. 10, A & C). This was especially true on the spore suspension-transfer

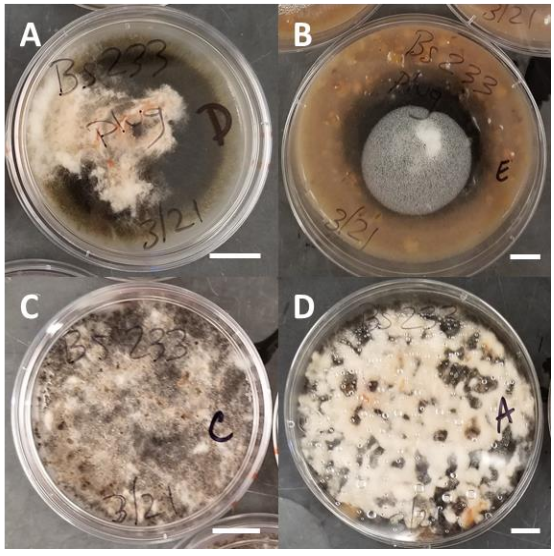

Figure 10. Growth of *B. sorokiniana* after eight days under black light on different media types: V8 agar (A and C) and V8 barley agar (B and D) using two different transfer types: plug (A and B) and spore suspension (C and D). Scale bars represent 1 cm.

plates.

The treatment using V8 agar and spore suspension-transfer had the highest average spore production at 4.3 x 10^4 spores/cm^2 (Fig. 11). The technique with the lowest spore production was plug-transfer onto V8 agar, resulting in an average production of 2.0 x 10^4 spores/cm^2. Both transfer methods onto V8 barley agar produced an average of 2.7 x 10^4 spores/cm^2 (Fig. 11). Variability in spore production for each combination of methods was high, with standard deviations ranging from 1.1 x 10^4 spores/cm^2 (V8 agar, plug-transferred) to 1.9 x 10^4 spores/cm^2 (V8 agar, spore suspension-transferred) (Fig. 11).

From the ANOVA, media type, transfer method, and their interaction were all found not to have statistically significant effects on spore production.

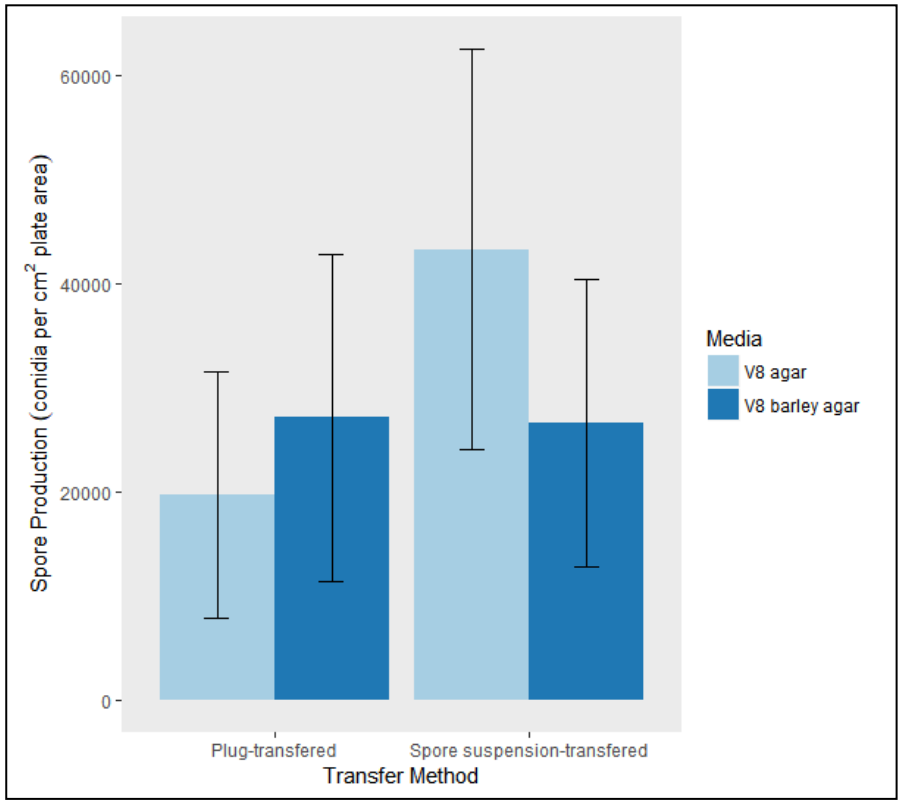

Figure 11. The average spore production (conidia/cm^2) of each media type and transfer method combination. Error bars represent a single standard deviation above and below the mean.

Comparing media, light source, and transfer method in producing Bipolaris sorokiniana inoculum

Total spore production

The combination of methods that resulted in the most conidia produced per plate was plug-transferring *B. sorokiniana* onto PDA++ and growing the plates in the dark. On average, this produced 1.9 x 10^6 spores per plate (Fig. 12). Because of fairly high variability, this was not found to be statistically significantly different from plug-transferring the fungus onto V8 and growing it under fluorescent light, which resulted in the production of an average of 1.4 x 10^6 spores per plate.

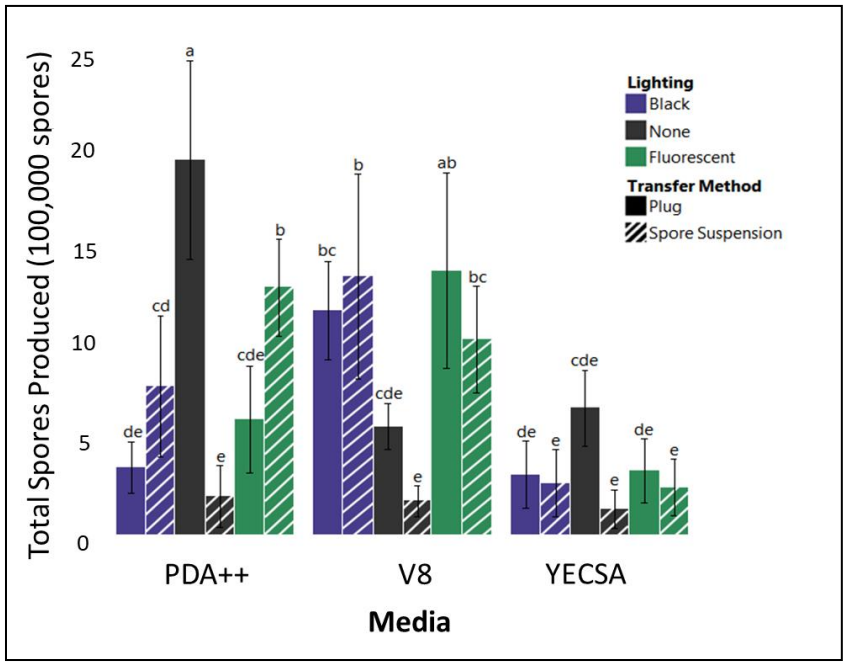

Figure 12. Average spore production for each media, spore transfer technique, and lighting source combination, in 8.5 cm diameter Petri dishes. Error bars represent a single standard deviation above and below the mean. Groups labeled with the same letter do not statistically differ, *P* =

On average, using YECSA produced far fewer spores than either of the other two media types. Using YECSA reduced spore production by an order of magnitude (Fig. 12; Table 43). Except for on PDA++, using either of the two light sources was better at inducing spore production than growing them in the dark (Fig. 12, Table 6). It was possible to find equally unsuitable conditions for producing *B. sorokiniana* conidia on all three media types by using spore suspension-transfer, and growing the plates in the dark (Fig. 12).

Table 43. Mean spore counts on each media type.

Media		Mean Spore Count
V8	a[x]	904,058
PDA++	a	819,604
YECSA	b	28,611

[x] Groups followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 44. Mean spore counts from each transfer technique.

Transfer Technique		Mean Spore Count
Plug	a	807,676
Spore suspension	b	601,049

[x] Groups followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 45. Mean spore counts for each light source.

Light Source		Mean Spore Count
Fluorescent	a	818,782
Black	a	728,500
None	b	462,491

[x] Groups followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Looking at the main effects on their own, the best combination of methods to produce as many conidia as possible would be use plug-transfer to start new plates (Table 44), to use V8 or PDA++ as the media type (Table 43), and to grow the spores under black or fluorescent light (Table 45). While it is informative to look at all of the main effects separately, these numbers do not take interaction effects into account and can therefore be misleading. From the ANOVA, all

effects and their interactions were found to be significant except for media type × transfer technique interaction (Table 46).

Table 46. Significance of main effects and the interaction terms from the ANOVA run on the total spore production.

Main effect	**Level of significance** [k]
Media	***
Transfer Technique	***
Light Source	***
2-Way Interaction	
Media × Transfer Technique	n.s.
Media × Light Source	***
Transfer Technique × Light Source	***
3-Way Interaction	
Media × Transfer Technique × Light Source	***

[k] n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p<0.001$

Average spore length

The combination of techniques that produced the longest conidia was plug-transferring the fungus onto V8 agar, and growing it under black light (Fig. 13). Other methods that were found not to be statistically different from this optimal set of conditions was to use either transfer technique onto V8 agar and then grow the fungus under fluorescent light, or to plug-transfer onto either PDA++ or YECSA and then grow the *Bipolaris* under black light (Fig. 13). The single factor that had the largest negative effect on conidia length, regardless of media type, was to grow the cultures in the dark. Growing the plates in the dark reduced spore length by 23% (Table 49). Growing *B. sorokiniana* on PDA++ also decreased the length of the spores by 17% from growing it on YECSA, and decreased spore length by 30% from growing it on V8 agar (Table 47). Using spore suspension-transfer decreased spore length by 15% (Table 48). The only effect

that was not found to be statistically significant in the ANOVA was the media type × light source interaction (Table 49).

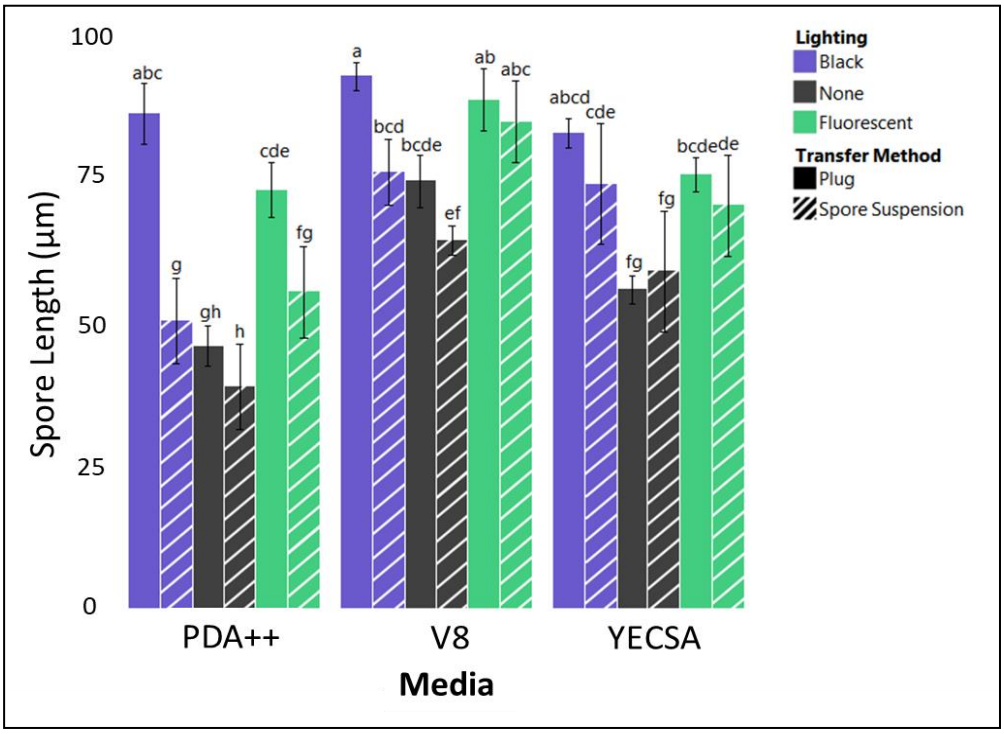

Figure 13. Average spore length for each media, spore transfer technique, and lighting source combination. Error bars represent a single standard deviation above and below the mean. Groups labeled with the same letter do not statistically differ, $P = 0.05$

Table 47. Mean spore length on each media type.

Media		Mean Spore Length (µm)
V8	a[x]	78
YECSA	b	68
PDA++	c	54

[x] Groups followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 48. Mean spore length from each transfer technique.

Transfer Technique		Mean Spore Length (µm)
Plug	a[x]	74
Spore suspension	b	63

[x] Groups followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 10. Mean spore length for each light source.

Light Source		Mean Spore Length (µm)
Black	a[x]	73
Fluorescent	a	72
None	b	55

[x] Groups followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 50. Significance of main effects and the interaction terms from the ANOVA run on the average spore length.

Main effect	Level of significance [k]
Media	***
Transfer Technique	***
Light Source	***
2-Way Interaction	
Media × Transfer Technique	***
Media × Light Source	n.s.
Transfer Technique × Light Source	***
3-Way Interaction	
Media × Transfer Technique × Light Source	*

[k] n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p<0.001$

Water volume absorbed by mycelium

The least amount of water absorbed by mycelium was on plates started with spore suspension-transfer and grown on V8 agar under fluorescent lighting (Fig. 14). Other methods were found not to be statistically significantly different from this optimal set of conditions for

producing little mycelium. These include all of the V8 agar and PDA++ plates that were plug-transferred. Very little mycelium was produced on any of the V8 agar plates, but abundant mycelium was produced on YECSA plates and PDA++ plates started with spore suspension (Fig. 14 & 14a).

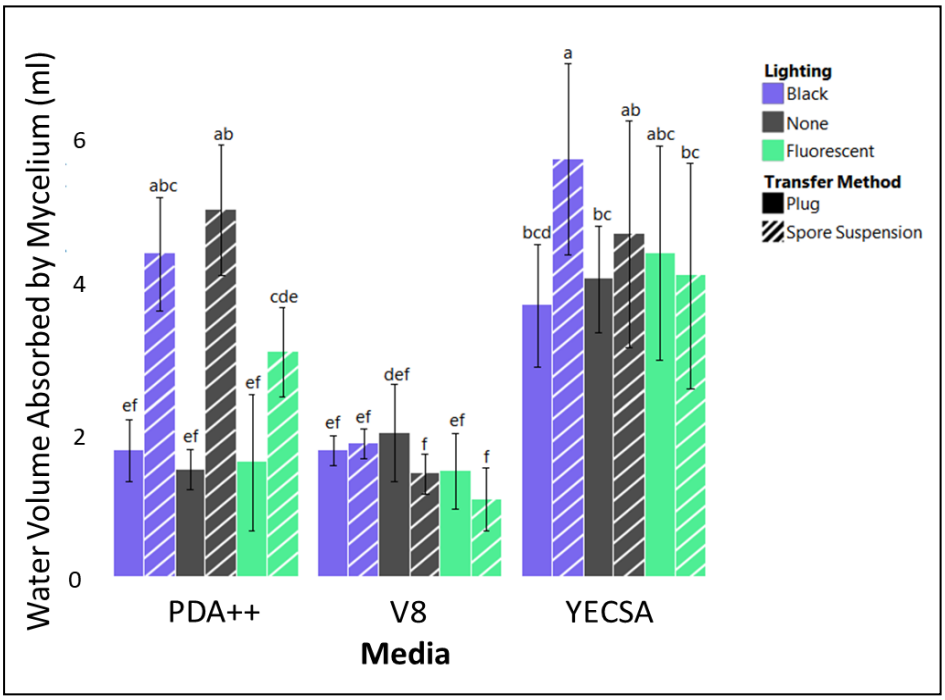

Figure 14. Average water absorbed by mycelium for each media, spore transfer technique, and lighting source combination. Error bars represent a single standard deviation above and below the mean. Groups labeled with the same letter do not statistically differ, $P = 0.05$

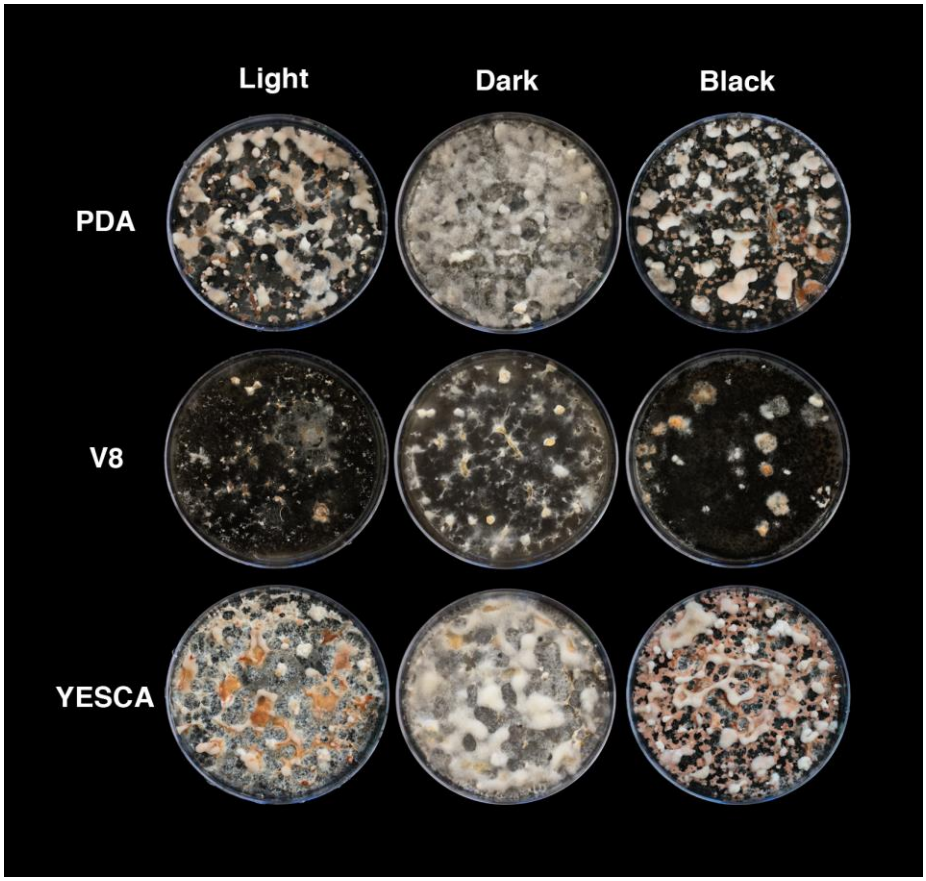

Figure 14a. Surface of spore suspension-started plates that were grown for ten days on three different media types: PDA, V8 agar, and YECSA, and under three different light sources: fluorescent (Light), no light (Dark) and black light (Black). Vegetative growth is orange, gray, and white. Reproductive growth is black.

Overall, YECSA-grown cultures absorbed the most water (Table 51), and therefore produced the most mycelium. In general, plates made with spore suspension-transfer (Table 52) and plates grown under black or no light (Table 53) produced the most mycelium. The only effects not found to be statistically significant from the ANOVA were the media type × light source interaction, and the interaction of all three effects (Table 54).

Table 51. Mean water absorbed on each media type.

Media		Mean Volume Absorbed (mL)
YECSA	a[x]	4.6
PDA++	b	3.3
V8	c	1.5

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 52. Mean water absorbed from each transfer technique.

Transfer Technique		Mean Volume Absorbed (mL)
Spore suspension	a[x]	3.5
Plug	b	2.5

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 53. Mean water absorbed for each light source.

Light Source		Mean Volume Absorbed (mL)
Black	a[x]	3.5
None	a	3.3
Fluorescent	b	2.7

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD test

Table 54. Significance of main effects and the interaction terms from the ANOVA run on the water absorbed by mycelium.

Main effect	Level of significance [k]
Media	***
Transfer Technique	***
Light Source	***
2-Way Interaction	
Media × Transfer Technique	***
Media × Light Source	n.s.
Transfer Technique × Light Source	**
3-Way Interaction	
Media × Transfer Technique × Light Source	n.s.

[k] n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p<0.001$

Conclusions

From the first experiment, none of the treatments were statistically different from each other, indicating that adding the host substrate to the media does not induce *B. sorokiniana* to sporulate. From the second experiment, it was found that transfer technique, media type, and light source can all have a dramatic effect on how many spores are produced, and on the morphology of the colony and spores. In terms of the pure number of spores, plug-transfer onto PDA++ and grown in the dark was the most effective combination of techniques, but the size of the spores produced was also the smallest. The combination of methods that produced the most and longest conidia, and the least amount of mycelium was plug-transfer of the fungus onto V8 agar grown under fluorescent light. The paucity of spores grown on YECSA under fluorescent light brings into question how this set of methods has been found to be successful for other laboratories. There was only one isolate tested in this study, and it is possible that different isolates could grow differently under these conditions.

The information that is clearly missing from this study is whether the different spore production techniques change the disease-causing ability of the conidia produced. This would require repeating the experiment and inoculating plants with inoculum produced by each combination of techniques. There are also more published methods that have yet to be compared to those trialed in this study

CHAPTER 4

USE OF A DIVERSE BARLEY POPULATION FROM THE UNIVERSITY OF MINNESOTA TO EXPLORE GENETIC RESOURCES FOR DISEASE RESISTANCE IN NEW YORK

Introduction

Barley production in New York is at risk from a variety of fungal diseases, all of which can cause significant losses if not controlled (Chapter 1 & 2). One of the key ways to control disease is through the use of disease-resistant cultivars. Identifying genetic sources of resistance to the pathogens in the state can aid in breeding new lines that are adapted to New York environments. One way to identify the genetic loci conferring the resistance is through genetic mapping and association studies.

Linkage mapping uses related individuals to find sections of the genome that segregate with the trait of interest. In plants, biparental populations are the most common populations used for linkage mapping (Jannink et al. 2001). Association studies rely on diverse populations that naturally have variation in the genome. The individuals in the diverse population are phenotyped and genotyped, and each marker is tested for association with the trait of interest (Risch 2000). The main benefits of genome-wide association studies in relation to linkage mapping are two-fold. One is that they can be used to analyze more allelic diversity than is available in a biparental population (Korte and Farlow 2013). The second is that they tend to have higher resolution because there has been more opportunity for recombination events in these populations (Korte and Farlow 2013). In extremely diverse populations, such as those with individuals from geographically diverse landraces or wild relatives of a cultivated species, these recombination events can be ancient (Roy et al. 2010).

Both types of mapping have been used to identify disease resistance quantitative trait loci (QTLs) in barley. For spot blotch, caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*), one of the first QTL linkage mapping studies was done on a population made from the doubled-haploid progeny of a cross between Steptoe and Morex, both six-row spring barley cultivars (Steffenson et al. 1996). In this study, one QTL for seedling resistance to spot blotch was found on chromosome 7H, and adult resistance QTLs were found on chromosomes 7H and 1H (Steffenson et al. 1996). Bilgic et al. (2006) screened a biparental population from a cross between a Mexican line and Bowman with barley yellow dwarf virus (BYDV) resistance introgressed. Quantitative trait loci for spot blotch were found on chromosomes 2H, 3H and 6H. Spot blotch resistance has also been mapped in wild barley. Roy et al. (2010) used association mapping to find 13 QTLs, seven of which were novel, when 318 accessions of *Hordeum vulgarum* subsp. *spontaneum* were screened with spot blotch. Haas et al. (2016) screened a biparental advanced backcross population from a cross between the wild accession PI 466423 as the donor parent and Rasmussen as the recurrent parent. Different QTLs were found depending on the *B. sorokiniana* isolate used, and depending whether the plants were seedlings or adults when screened (Haas et al. 2016). The most recent publication on QTL mapping for barley spot blotch resistance is Wang et al. (2017) where the diverse USDA core collection of barley was screened as seedlings using three different pathotypes of *B. sorokiniana*. Again, different QTLs were found depending on the pathotype used in the screen.

The other two diseases used to evaluate barley varieties for resistance in this study, powdery mildew, caused by *Blumeria* f. sp. *hordei*, and leaf rust, caused by *Puccinia hordei*, are both biotrophs. As would be expected for a biotrophic pathogen-plant host interaction, there are

several major resistance genes that have been identified for both diseases, but QTL mapping studies have also been used to find more durable and diverse types of resistance.

The most common sources of powdery mildew resistance used in barley are *Mla* and *mlo*. The *Mla* locus is a complex of about 30 tightly-linked NBS-LRR genes that can confer race-dependent resistance to powdery mildew (reviewed in Jørgensen 1994; Wei et al. 2002). The *Mla* region is located around 38 cM from the end of the short arm on chromosome 1H (Ames et al. 2015). Several other race-specific resistance genes can be found on chromosome 1H, and elsewhere in the genome (reviewed in Jørgensen 1994). The other common source of resistance to powdery mildew is *mlo*, located on chromosome 5H. This locus does not confer race-specific resistance, and has proven to be extremely durable. Lines carrying the recessive allele at *Mlo* block cell wall penetration, but the exact function of the gene is not yet known (Acevedo-Garcia et al. 2014). Quantitative trait locus mapping has revealed other resistance loci. Spies et al. (2012) discovered 11 QTLs that were race-non-specific on every chromosome except for 6H. Li and Zhou (2011) used two doubled-haploid populations to find five QTLs for powdery mildew resistance. Some studies have also looked to wild barley to find novel sources of resistance. Backes et al. (2003) created recombinant inbred lines from a cross between the two-row spring barley cultivar, Vada, and an accession of *H. vulgare* subsp. *spontaneum*, and found five powdery mildew resistance QTLs. Ames et al. (2015) screened 316 accessions from the Wild Barley Diversity Collection, and, using association mapping, found 15 quantitative trait loci, seven of which were novel.

There are 23 loci conferring leaf rust resistance that have been formally identified and named with the prefix *Rph* (Ziems et al. 2017). Some of these loci have only been identified in *H. vulgare* subsp. *spontaneum*, and *H. bulbosum* (Park et al. 2015). These leaf rust resistance loci

range in the degree of resistance they provide, and whether they provide it at the adult stage or seedling stage (Park et al. 2015). Only two of these loci are known to provide adult plant resistance. The other 21 have only been characterized as providing resistance at the seedling stage (Ziems et al. 2017). A doubled-haploid mapping population designed to map *Rph20*, a locus conferring partial adult plant resistance, identified an additional six loci associated with adult plant resistance across five environments (Hickey et al. 2011). An association study using three biparental populations that reported three QTLs were significantly associated with adult plant resistance in each population (Singh et al. 2017).

The purpose of this study was to use a population of two-row spring barley made up of breeding lines from five different breeding programs, and their crosses, to find QTLs for disease resistance. Seedlings of the population were screened with an aggressive isolate of *B. sorokiniana* collected in New York, and adults were rated for other naturally-occurring diseases in New York.

Methods

Plant population

The two-row spring barley population used for disease evaluations was developed at the University of Minnesota as a tool for genomic selection (Neyhart 2015). It is a combination of a selection of the lines from a training population (S2TP) and a cycle 1 selection population (C1). The S2TP is composed of 183 lines from five U.S. breeding programs, including USDAA/ARS (Aberdeen, ID), Montana State University, Washington State University, Busch Agricultural Resources, and North Dakota State University. The C1 population was composed of inbred lines from bi-parental crosses between selected lines in the S2TP (Neyhart 2015). The population

evaluated in this study is composed of 183 lines from the TP and 50 lines from the C1. The 233 lines are collectively called the MET population.

All of the lines in the S2TP and C1 were genotyped using genotyping-by-sequencing (Elshire et al. 2011), and the single nucleotide polymorphisms (SNPs) were mapped to their genetic and physical locations (Mascher et al. 2017) in the barley genome. A SNP marker was removed if more than 20% of the data were missing. There were 11,039 bi-allelic SNP markers reported for the population. (Neyhart, unpublished data). These marker data will be made publicly available on the T3/Barley database (https://triticeaetoolbox.org/barley/).

Greenhouse evaluation of spot blotch seedling resistance

Experimental design

Eight repetitions of the spot blotch barley evaluation were performed. Each repetition consisted of a single replicate of the MET in a completely randomized design. Five check cultivars, chosen for their diverse response to spot blotch (Chapter 3), were randomly interspersed within the MET. The resistant checks were AAC Synergy and Newdale and the susceptible checks were Full Pint and Craft. Conlon was included because it has been found to have an intermediate response to spot blotch. For each of the checks, three replicates treated with the inoculum and three non-treated replicates were included per repetition of the screen.

Plant material

Each line was planted three seeds to a pot, using 4 inch-diameter pots. The plants were grown in a soilless medium, Cornell Potting Mix (3.8 bales peat moss, 9.1 kg vermiculite, 9.1 kg perlite, 2.3 kg lime, 1.8 kg Jack's Professional 10-5-10 Media Mix Plus III (JR Peters Inc, Allentown, PA), 0.11 kg AquaGro® wetting agent (Aquatrols, Paulsboro, NJ)). The plants were grown in the greenhouse (22ºC, 12-hour light cycle, watered once daily with tap water (0.34

mg/L chlorine) with 300 ppm nitrogen added twice weekly). At least one day before inoculation, excess seedlings were removed so that there were only two plants per pot. Seedlings were inoculated 15 days after planting, at approximately the second leaf stage.

Bipolaris sorokiniana production

The *Bipolaris sorokiniana* isolate used in this screen was Bs233NY15, collected in 2015 from Conlon grown in Delaware County, NY (Chapter 3). A stock plate was made by plating the isolate from its glycerol stock, stored at -80ºC, directly onto V8 agar (163 mL V8 juice, 2.44 g $CaCO_3$, 12.2 g agar, 650 mL DI H_2O). After ten days of growth under black light (12-hour photoperiod), these plates were used to make a highly-concentrated spore suspension. New plates were created by pipetting 75 µL of the spore suspension onto each V8 plate and spreading the suspension using a sterilized L-shaped glass rod. The plates were grown under black light (40W; 350 nm peak wavelength; 12-hour photoperiod) for ten days before the spores were collected for use in inoculum.

Inoculum production

After the plates of *B. sorokiniana* had grown for 10 days, the spores were collected by flooding each plate with 10 mL water and gently scraping the surface of the agar with an L-shaped glass rod. The spore suspension was filtered through three layers of cheesecloth. Spores were counted using a hemocytometer, and the concentration of the spore suspension for each isolate was adjusted to 3.4×10^4 cells/mL. The surfactant, TWEEN20 (polysorbate 20) was added to the spore suspension (100ul/L). The control solution was made using H_2O and TWEEN20 (100ul/L).

Inoculation

Seedlings were inoculated when the second leaf was fully emerged. The second leaf was sprayed with a fine mist of inoculum to achieve maximal coverage of the leaf surface with fine droplets without causing coalescence of the droplets or runoff of suspension using Preval® aerosol sprayers (Chicago Aerosol, Coal City, IL). The plants were allowed to dry before being moved. Once the leaves were dry, the pots were moved into the mist chamber (set to 22ºC, 90% humidity, with no additional lighting). After the plants had been in the mist chamber for 17 hours, the mist chamber was switched off and the doors were opened to allow the plants to dry again. Once the plants had dried, the pots were moved back into the greenhouse.

Rating

Ten days post-inoculation, ratings were taken as a visual estimate of the percent leaf area covered by lesions on the second leaf. The spot blotch severities of the two plants in each pot were averaged, resulting in a single severity score per pot.

Leaf rust and powdery mildew ratings on adult-stage MET in the field

Barley cultivation

In 2016, two replicates of the MET were planted at the Caldwell Research Field in Ithaca, NY (Tompkins County) in a randomized complete block design. Each replicate included every line of the MET, and seven check cultivars, each with three entries per replicate of the MET. In 2016, the field was prepared with a 336 kg/ha pre-plant application of 10:20:20 fertilizer (delivering 33.6 kg/ha of nitrogen). A mixture of Maestro 2EC (bromoxynil, Nufarm, Melbourne, Australia) and Harmony Extra SG (a mixture of thifensulfuron methyl and tribenuron methyl, DuPont, Newark, DE) with Induce (nonionic surfactant, Helena Chemical, Collierville, TN) was applied in early May. Each line was planted in a single, 1-m row, on 15

April 2016. This planting was regularly misted after heading to encourage the development of Fusarium head blight (FHB).

In 2017, the MET was planted at two fields, Helfer and Ketola, in Ithaca, NY (Tompkins County). One full replicate was planted in a completely randomized design at each location. At each location, three replicates of seven check cultivars were randomly interspersed within the MET lines. Each line was planted in a 4-m-long plot that was 6 rows wide with 18 cm row spacing. Seed was planted at a rate of 107.6 kg/ha on 26 April at Ketola and on 1 May at Helfer. Fields were prepared with a 134.5 kg/ha application of 27:18:9 fertilizer, delivering 35.9 kg/ha of nitrogen. In early May, a broadleaf herbicide with the same active ingredients as in 2016 was applied. No fungicides or insecticides were applied, and no artificial inoculations of diseases were performed for any of the MET field trials.

Disease ratings

Disease ratings were taken by estimating the percent leaf area of the top two leaves damaged by the symptoms and signs of each disease. Leaf rust data were recorded on July 12 in 2016 while the barley was in the ripening growth stage (Zadoks' growth stage 90). Leaf rust and powdery mildew data were recorded on 11 July at the Helfer field, and on 13 July at the Ketola field, while the barley was at the hard dough growth stage (Zadok's growth stage 87).

Statistical analysis

Genotypic data

Genotypic data were missing for ten members of the MET, so they were removed from the analysis. The SNP markers were screened for minor allele frequency (MAF) of less than 5%, and 2,157 SNPs that fell below this threshold were removed. All remaining 223 barley lines and 8,882 SNP markers were analyzed in each genome-wide association (GWA) analysis.

Population structure

To account for the possibility that lines in the MET coming from the same breeding program have high genetic similarity, a principal component analysis (PCA) was performed on the 8,882 SNP markers with MAF > 0.05 for the 223 barley lines. The PCA was done using the 'prcomp' function found in the basic stats package in R (R Core Team 2017).

Association mapping

The check varieties of each screen were analyzed using ANOVA to verify that genotypic variability accounted for a statistically significant portion of the variance in disease severity. Variety, replicate, and their interaction were included in each ANOVA.

For the greenhouse screen of spot blotch on seedlings, the best linear unbiased estimator (BLUE) for each line's response to spot blotch was calculated using a mixed linear model with replicate as a random effect and line as a fixed effect. These values were used as the response variable in the seedling disease resistance GWA analysis.

Disease severity data for powdery mildew and leaf rust were square root-transformed, so that the data distributions were closer to normal. The average was taken of the square root-transformed disease severity scores from the two replicates of each screen. These values were used as the response variables in the adult disease resistance GWA analyses.

GWA analysis was performed on each screen using the rrBLUP package in R (Endelman 2011), which was based on the linear mixed model from Yu et al. (2006). Genotypic values were included in the model as random effects, and the first two principal components (PCs) were included in the model as fixed effects to account for population structure.

For each test, the QQ plot generated with the -log(*p*-values) was examined to ensure that it followed the pattern expected when thousands of statistical tests on variables that have no true

effect, as is the case for most of the SNPs in each GWA analysis. The *p*-values of non-significant statistical tests follow a uniform distribution, so the points in the QQ plot are expected to follow a line with a slope of 1 and an intercept of 0. The only portion of the plot where the -log(*p*-values) should deviate from this line is in the case that there are QTLs, in which case several markers will have highly-significant *p*-values, which is represented by a "tail" that rises above the 1:1 line at the right side of the plot. A QQ plot that does not follow this model indicates that there are some significant covariates of the phenotype, such as population structure, that have not been included in the model.

The linkage disequilibrium (LD), using r^2 as the metric, was calculated between the most significant SNP of each QTL and its neighboring SNP markers, to generate a potential interval in which the genetic basis for the QTL would likely be located. The cutoff for the r^2 value for two markers to be considered in LD was 0.2 (Roy et al. 2010).

Results

Population structure

The first two PCs of the PCA explained 17.1% of the variation in the genotypic data. When the first two PCs are plotted against each other, the pattern of the similarity within and between breeding program populations is clear (Fig. 15). The North Dakota germplasm was more similar to itself than any other breeding population, but the germplasm from the four other original breeding programs grouped together. The germplasm coming from Minnesota was derived from crosses of lines from the other breeding programs. This is depicted in the graph because the individuals from the University of Minnesota cluster at the center of all the other breeding programs (Fig. 15).

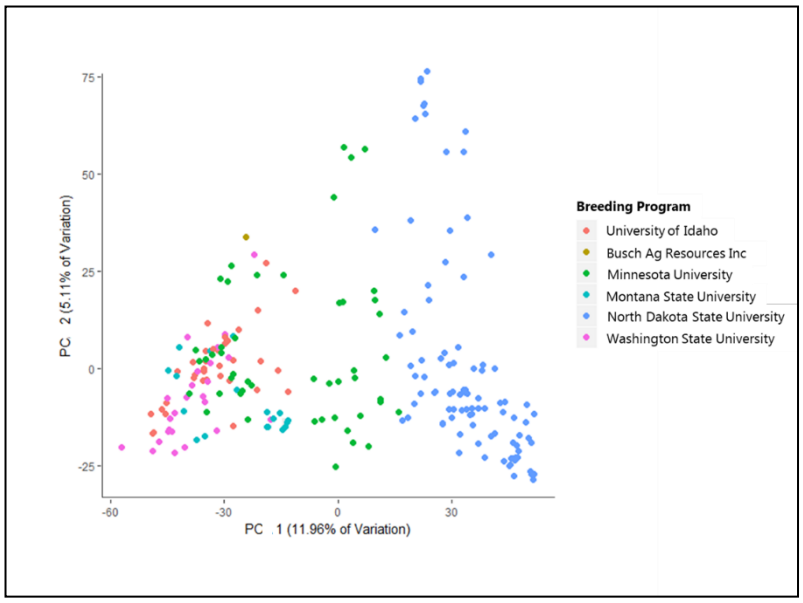

Figure 15. The first and second principal components (PCs) of the principal component analysis performed on 8,882 SNPs for the 233 members of the MET population. The first PC explained 11.96% of the variation in the genotypic data and the second PC explained 5.11% of the variation. The colored dots represent the population that the individual came from. The individuals from Minnesota University are the result of crosses made between members of the other populations.

Greenhouse screen of spot blotch on seedlings

Both variety and replicate were found to be highly significant in the ANOVA run on the check cultivars in the greenhouse spot blotch evaluation (Table 55). The checks also responded to the spot blotch evaluation as was expected (Table 56). Craft was the most susceptible, Full Pint was the second-most susceptible, Conlon fell at the center of the range of susceptibility, and Newdale and AAC Synergy clustered together as the most resistant varieties (Table 56).

Table 55. Significance of main effects and the interaction term from the ANOVA run on the spot blotch screen checks.

Effect	p-value	Level of significance[k]
Variety	8.88E-09	***
Replicate	4.06E-12	***
Variety × Rep	0.05136	n.s.

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Table 56. Mean spot blotch severities for the check cultivars, averaged across all replicates of the spot blotch screen.

Variety		Mean spot blotch severity (%)
Craft	a[x]	56.9
Full Pint	ab	43.3
Conlon	bc	27.5
Newdale	c	14.5
AAC Synergy	c	13.6

[x] Varieties followed by the same letter do not statistically differ, *P* = 0.05, Tukey's HSD

After averaging across the 8 replicates of the screen, the range of the seedling susceptibility to spot blotch in the MET extended beyond that of the check cultivars, with values from 0 to 70% spot blotch severity (Fig. 16). The BLUEs calculated for the spot blotch severities clustered more symmetrically around the mean (Fig. 17) than the averages (Fig. 16).

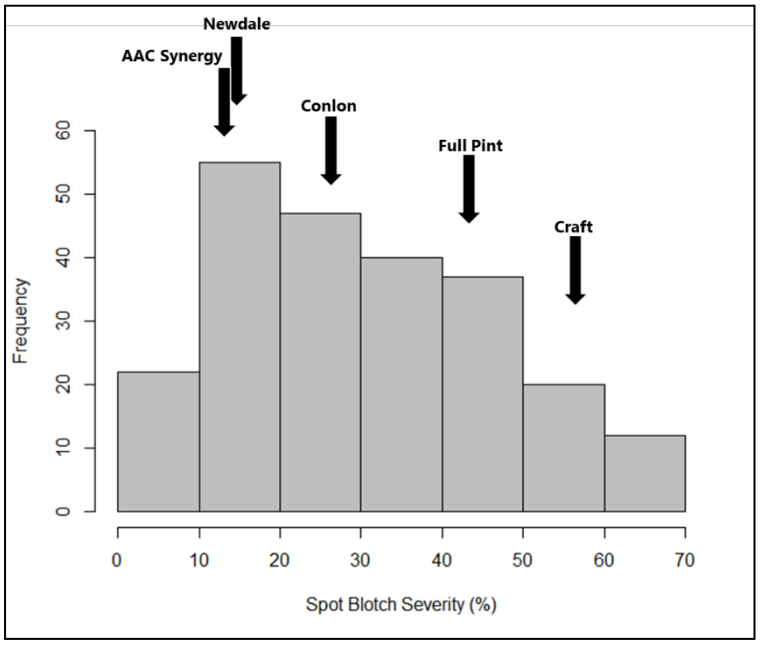

Figure 16. Histogram of the mean spot blotch severities of the 233 individuals in the MET population from the 8 repetitions of the screen. The black arrows point to the mean spot blotch severities of each of the five check varieties. All of the checks had the expected response to spot blotch.

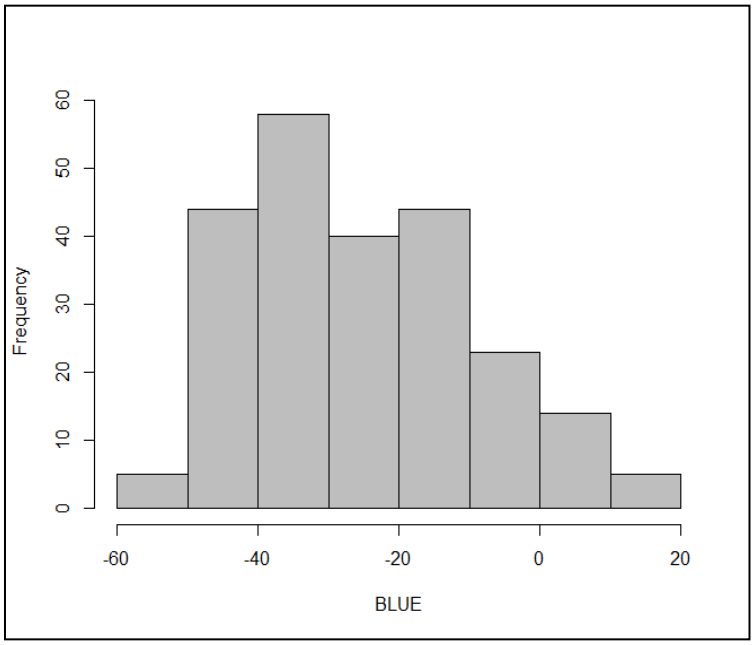

Figure 17. Distribution of the best linear unbiased predictors (BLUEs) of spot blotch severity of the 233 individuals in the MET population.

Two peaks were identified in the GWA analysis for spot blotch (Fig. 18). One was located on the long arm of chromosome 2H, with two SNP markers found to be significant, and the other was located on the short arm of chromosome 7H, with one marker found to be significant (Fig. 18, Table 57). The three significant markers explained between 3.1% to 35.4% of the phenotypic variation and had allelic effects ranging from 1.4% to 16.4% (Table 57). The PCs included in the model adequately accounted for the population structure in the genetic data (Fig. 19).

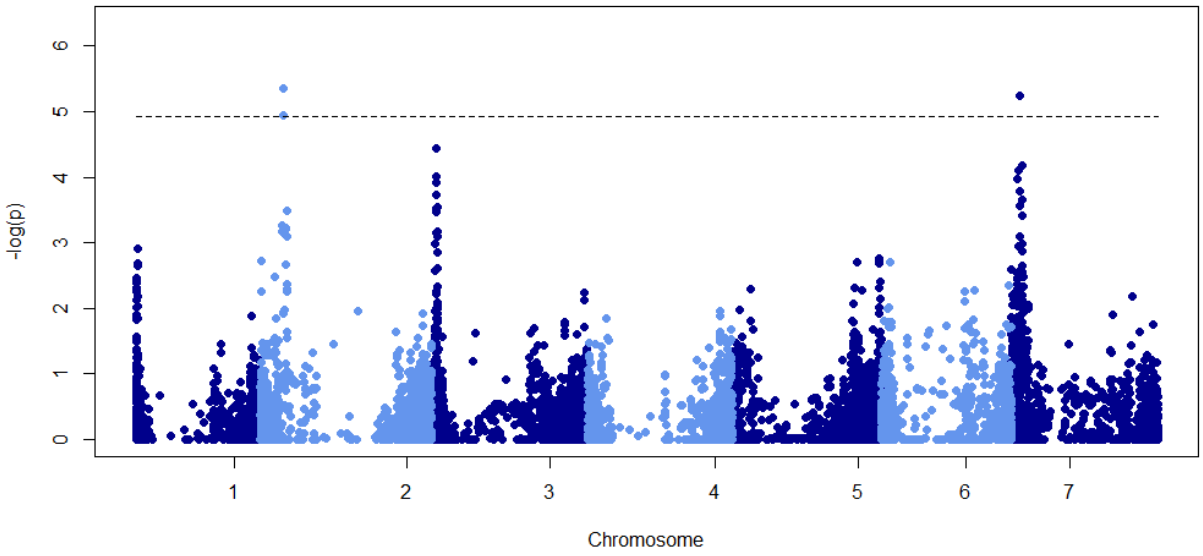

Figure 18. Manhattan plot of the –log(*p*-values) calculated for 8,882 SNPs, mapped to their position in the barley genome. The p-values were obtained from the genome-wide association study performed on the spot blotch severity scores of 223 two-row spring barley lines screened with *B. sorokiniana* isolate Bs233NY15. Plants were rated for disease at the seedling stage. The dashed line represents a false discovery rate cut-off of 5%. Only the points above the line are considered to be statistically significant.

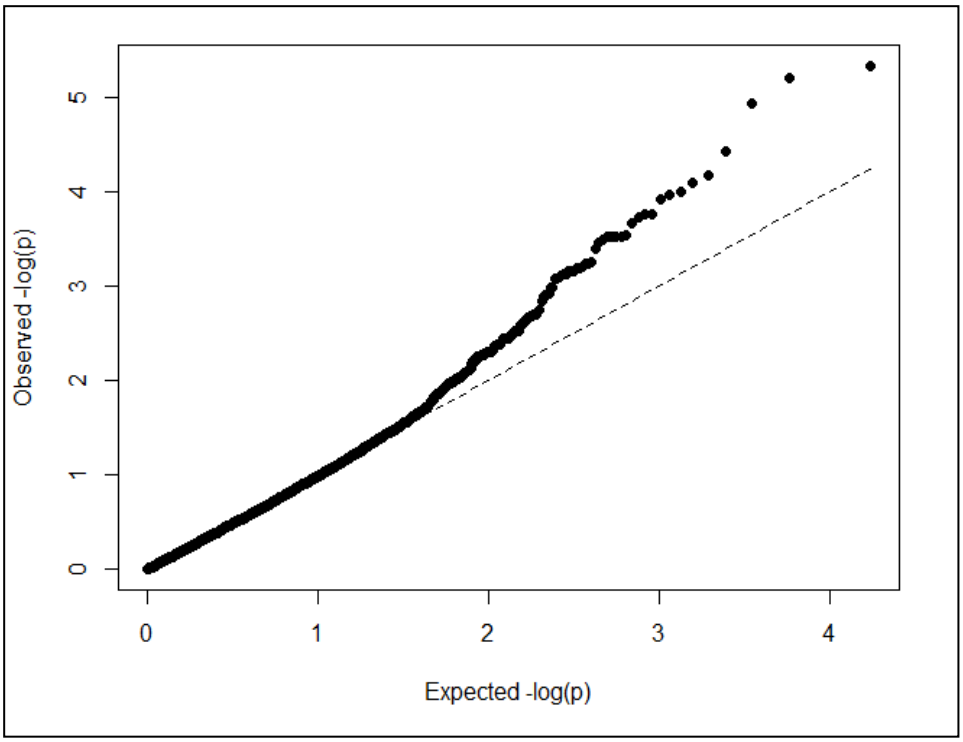

Figure 19. QQ plot of the –log(*p*-values) for 8,882 SNPs obtained from the genome-wide association analysis performed on the spot blotch response of 223 members of the MET population. The structure of the population was adequately accounted for as is demonstrated by the plotted –log(*p*-values) matching the 1:1 line, representative of the uniform distribution of *p*-values expected when no markers are significantly associated with the disease. The tail at the right-hand side of the graph is a result of the highly-significant *p*-values found for SNPs associated with disease resistance.

Table 57. Description of significant markers in the spot blotch GWA analysis

Marker	Alleles	Chromosome	Position	cM Position	-log(p)	p-value	R^2 (%)[n]	Allele Effect (%)[m]	Resistant Allele[p]
S2_100387796	T/C	2H	100387796	69.86	4.95	1.12E-05	4.2	16.4	T
S2_100387825	G/A	2H	100387825	69.86	5.35	4.47E-06	3.1	1.4	G
S7_39314906	G/A	7H	39314906	33.62	5.21	6.17E-06	35.4	11.8	G

[m] Allele effect = (the mean of infection responses of lines carrying susceptible alleles − the mean of infection responses of lines carrying resistant alleles) / the mean of infection responses of lines carrying susceptible alleles *100%
[n] The marker R^2 is the portion of phenotype variance explained by the marker, with no other terms included in the model
[p] Allele of the SNP marker found to be carried by the more-resistant individuals

The markers in LD with the marker with peak significance for the QTL on chromosome 2H (69.86 cM) range from S2_93818718 (60.09 cM) to S2_119984505 (73.16 cM) on chromosome 2H, representing a span of 26.2 Mbp or 4.07 cM (Fig. 20). This section of the chromosome is a less-conservative estimate of the QTL's location. The markers just beyond this range of LD are S2_93387329 (69.09 cM) and S2_120720623 (73.60 cM), representing a span of 27.0 Mbp or 4.51 cM. This section of the chromosome is a more-conservative estimate of the QTL's location.

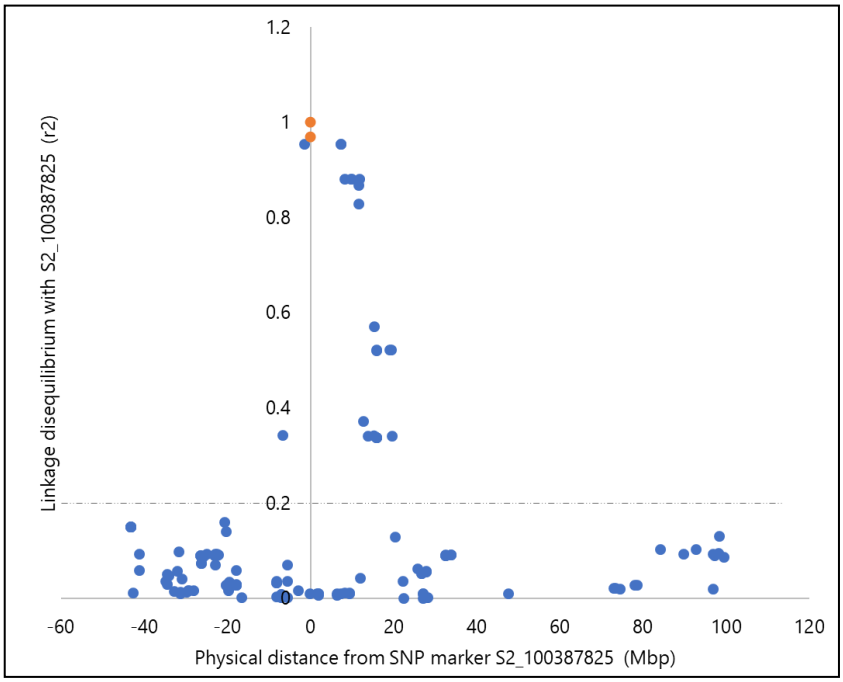

Figure 20. Plot of the linkage disequilibrium (LD) of the 65 markers located directly upstream and downstream of S2_100387825, the most significant marker found for seedling resistance to spot blotch on chromosome 2H. The dashed line is drawn at $r^2 = 0.2$, which was the cutoff for points to be considered in LD with the marker. Only the orange points correspond with markers (S2_100387825 itself and S2_100387796) found to be significant in the GWA analysis. The SNPs marking the boundaries of LD with S2_100387825 are S2_093818718 and S2_119984505, representing a span of 26.2 Mbp.

The markers considered to be in LD with the marker with peak significance for the QTL on chromosome 7H (33.62 cM) range from S7_27771398 (28.86 cM) to S7_40776110 (35.07 cM), representing a span of 13.0 Mbp or 6.21 cM (Fig. 21). The markers just beyond this range of LD are S7_27544028 (28.71 cM) and S7_40927464 (35.21 cM), representing a span of 13.4 Mbp or 6.5 cM (Fig. 21).

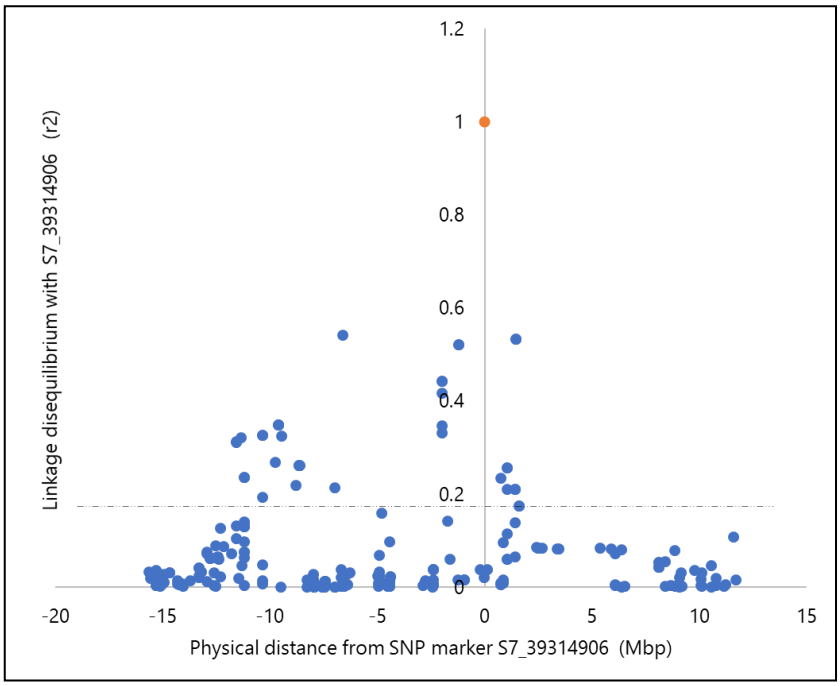

Figure 21. Plot of the linkage disequilibrium (LD) of the 160 markers located directly upstream and 65 markers downstream of S7_39314906, the most significant marker found for seedling resistance to spot blotch on chromosome 7H. The dashed line is drawn at $r^2 = 0.2$, which was the cutoff for points to be considered in LD with the marker. Only S7_39314906 itself, the orange point, was found to be significant in the GWA analysis. The SNPs marking the boundaries of LD with S7_39314906 are S7_27771398 and S7_40776110, representing a span of 13.0 Mbp.

Powdery mildew in 2017 field trials

Variety was found to be significant in the ANOVA run on the powdery mildew severity scores from the MET field trial in 2017 (Table 58).

Table 58. Significance of main effects and the interaction term from the ANOVA run on the powdery mildew scores of the checks in the 2017 field MET trial

Effect	p-value	Level of significance[k]
Variety	0.03	*
Location	0.35	n.s.
Variety × Location	0.19	n.s.

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

The mean powdery mildew severities for the check cultivars were not significantly different from each other when tested using Tukey's HSD (α = 0.05) (Table 59).

Table 59. Mean powdery mildew severities for the check cultivars, averaged across the two replicates of the MET planted in the field in 2017

Variety		Mean powdery mildew severity (%)
Hockett	a[x]	5.9
ND Genesis	a	2.6
CDC Copeland	a	2.3
AAC Synergy	a	2.1
AC Metcalfe	a	2
Conlon	a	1
Conrad	a	0
LCS_Genie	a	0
Pinnacle	a	0

[x] Varieties followed by the same letter do not statistically differ, *P* = 0.05, Tukey's HSD

There were some lines in the MET that had mean powdery mildew severity scores higher than the check cultivars. One line reached an average 50% powdery mildew severity, which was the maximum severity observed on any line. It was not possible for lines to be more resistant

than Pinnacle, LCS Genie, or Conrad, which were observed to have no powdery mildew in either location (Fig. 22).

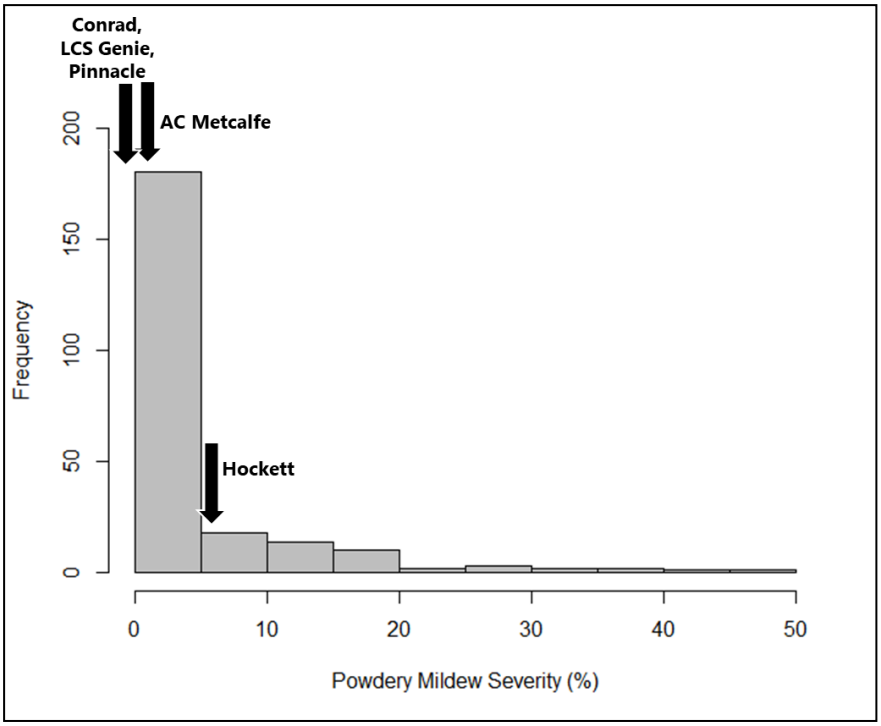

Figure 22. Histogram of the untransformed mean powdery mildew severities of the 233 individuals in the MET population from the two replicates of the screen planted in the field in 2017. The black arrows represent the range of disease observed in the checks by showing the mean powdery mildew severity of each of the check varieties with the highest, median, and lowest powdery mildew severity. There were seven check varieties planted.

One peak was identified in the GWA analysis for powdery mildew (Fig. 23). It was located on the short arm of chromosome 1H, with a single significant SNP marker (Table 60). The variation explained and the allelic effect of the single significant marker were 37.9% and 32.4%, respectively (Table 60). The PCs run in the model adequately accounted for the population structure in the genetic data (Fig. 24).

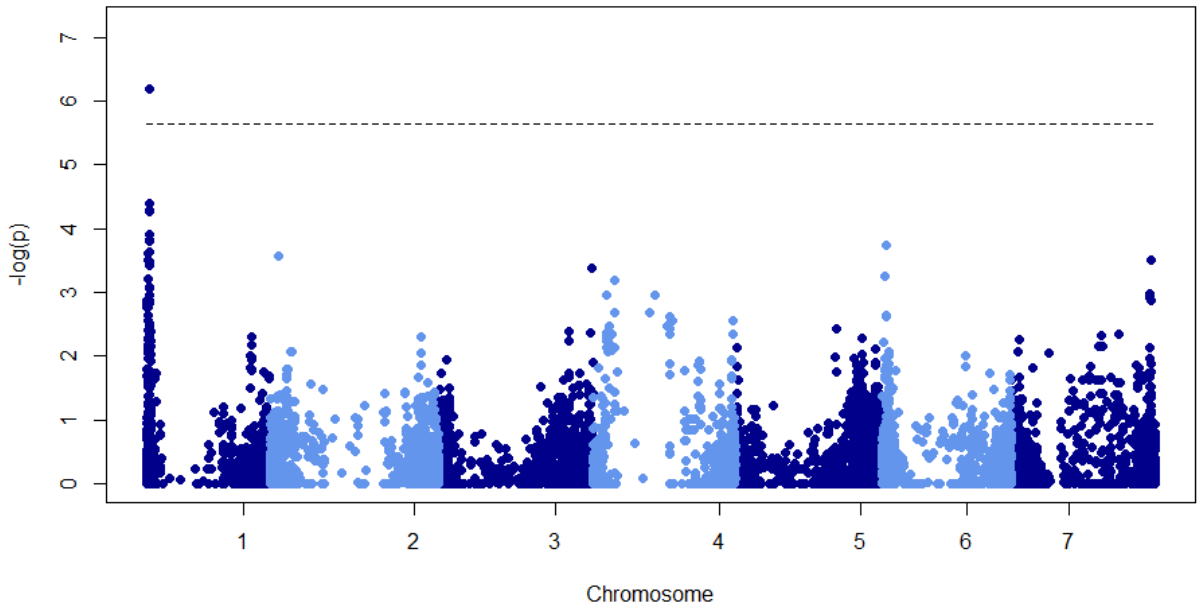

Figure 23. Manhattan plot of the –log(*p*-values) calculated for 8,882 SNPs, mapped to their position in the barley genome. The p-values were obtained from the genome-wide association study performed on the powdery mildew severity scores of 223 two-row spring barley lines infected with the disease from natural inoculum. Plants were rated for disease at the adult stage. The dashed line represents a false discovery rate cut-off of 5%. Only the points above the line are considered statistically significant.

Table 60. Description of significant markers in the powdery mildew GWA analysis

Marker	Alleles[f]	Chromosome	Position	cM Position	-log(p)	p-value	R^2 (%)[n]	Allele Effect (%)[m]	Resistant Allele[p]
S1_12034784	T/C	1H	12034784	6.12	6.19	6.46E-07	37.9	32.4	T

[m] Allele effect = (the mean of infection responses of lines carrying susceptible alleles − the mean of infection responses of lines carrying resistant alleles) / the mean of infection responses of lines carrying susceptible alleles *100%

[n] The marker R^2 is the portion of phenotype variance explained by the marker, with no other terms included in the model

[p] Allele of the SNP marker found to be carried by the more-resistant individuals

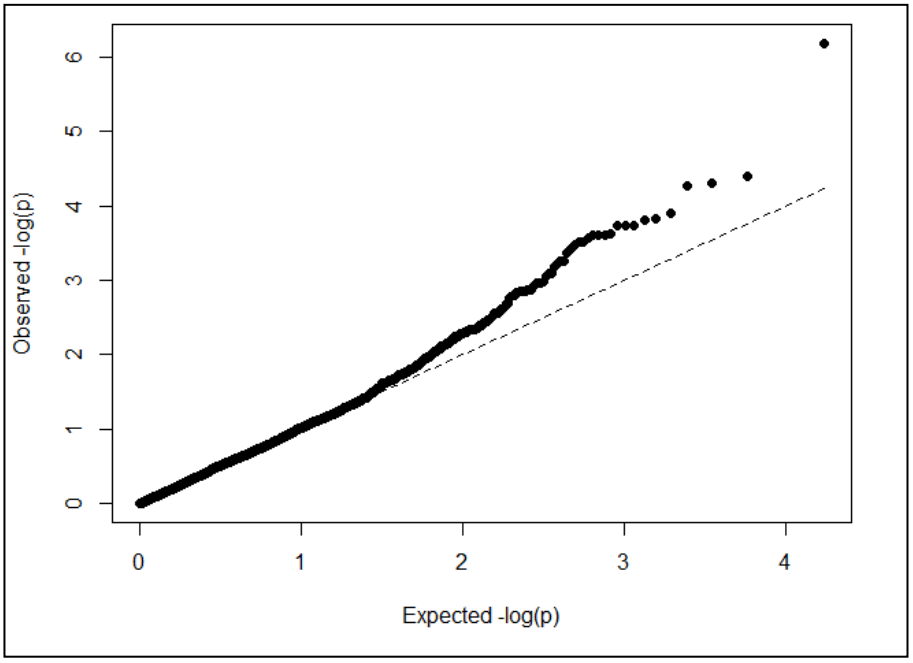

Figure 24. QQ plot of the –log(*p*-values) for 8,882 SNPs obtained from the genome-wide association analysis performed on the powdery mildew disease severity of 223 members of the MET population. The structure of the population was adequately accounted for as is demonstrated by the plotted –log(*p*-values) matching the 1:1 line, representative of the uniform distribution of *p*-values expected when no markers are significantly associated with the disease. The tail deviating from the 1:1 line at the right-hand side of the graph is a result of the highly-significant *p*-values found for SNPs associated with disease resistance.

The markers in LD with S1_12034784 (6.12 cM), the marker with peak significance for the powdery mildew QTL on chromosome 1H, range from S1_7824901 (4.85 cM) to S1_13306226 (6.79 cM), representing a span of 5.5 Mbp or 1.94 cM (Fig. 25). The markers directly flanking this range of LD are S1_7713768 (4.75 cM) and S1_13584219 (7.04), representing a span of 5.9 Mbp or 2.29 cM.

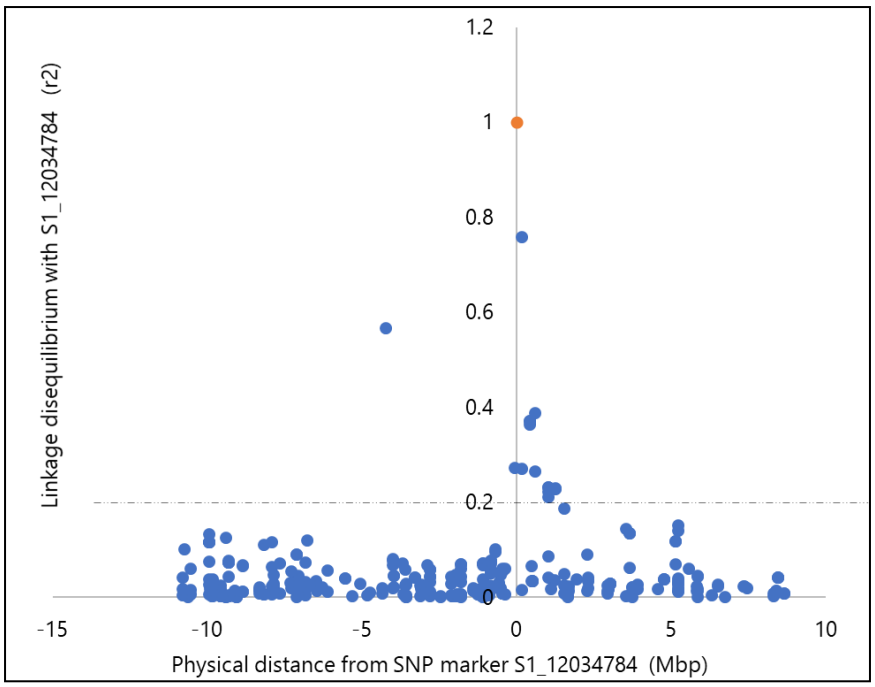

Figure 25. Plot of the linkage disequilibrium (LD) of the 209 markers located directly upstream and 100 markers downstream of S1_12034784, the most significant marker found for adult resistance to powdery mildew on chromosome 1H. The dashed line is drawn at r^2 = 0.2, which was the cutoff for points to be considered in LD with the marker. Only S1_12034784 itself, the orange point, was found to be significant in the GWA analysis. The SNPs marking the boundaries of LD with S1_12034784 are S1_7824901 and S1_13306226, representing a span of 5.8 Mbp.

Leaf rust in 2016 and 2017 field trials

Variety was found to be highly significant in the ANOVA run on the check cultivars' leaf rust severity scores in the 2016 planting of the MET (Table 61). Neither the replicate nor the variety × replicate interaction term was significant (Table 61). There was a range of responses to leaf rust in the check cultivars, from an average of 0.5% leaf rust severity in ND Genesis to 13.3% leaf rust severity in Hockett (Table 62). None of the MET individuals fell below this range, but several were more susceptible, with one line reaching 40%, the maximum severity observed (Fig. 26).

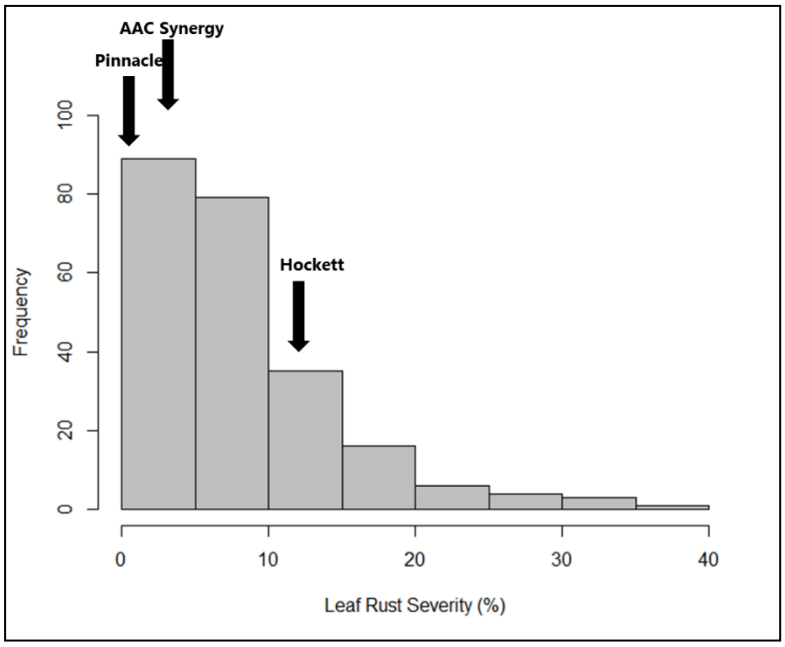

Figure 26. Histogram of the mean leaf rust severities of the 233 individuals in the MET population from the two replicates of the screen planted in the Fusarium head blight nursery in 2016. The black arrows represent the range of disease observed in the checks by showing the mean leaf rust severity of each of the check varieties with the highest, median, and lowest leaf rust severity. There were seven check varieties planted.

Table 61. Significance of main effects and the interaction term from the ANOVA run on the leaf rust scores of the checks in the 2016 field MET trial.

Effect	p-value	Level of significance[k]
Variety	0.0004	***
Replicate	0.9476	n.s.
Variety × Rep	0.9941	n.s.

[k] n.s. = not significant, * = p < 0.05, ** = p < 0.01, *** = p<0.001

Table 62. Mean leaf rust severities for the check cultivars, averaged across the two replicates of the MET planted in the field in 2016.

Variety		Mean leaf rust severity (%)
Hockett	a[x]	13.3
CDC Copeland	ab	11.2
Conlon	abc	9.2
Conrad	abc	6.2
AAC Synergy	bc	3.3
AC Metcalfe	bc	2.7
LCS Genie	bc	1.9
Pinnacle	bc	1.8
ND Genesis	c	0.5

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD

Variety and the variety × replicate interaction were found to be highly significant in the ANOVA run on the check cultivars' leaf rust severity scores in the 2017 planting of the MET, but replicate was not significant (Table 63). The checks ranked similarly to their leaf rust

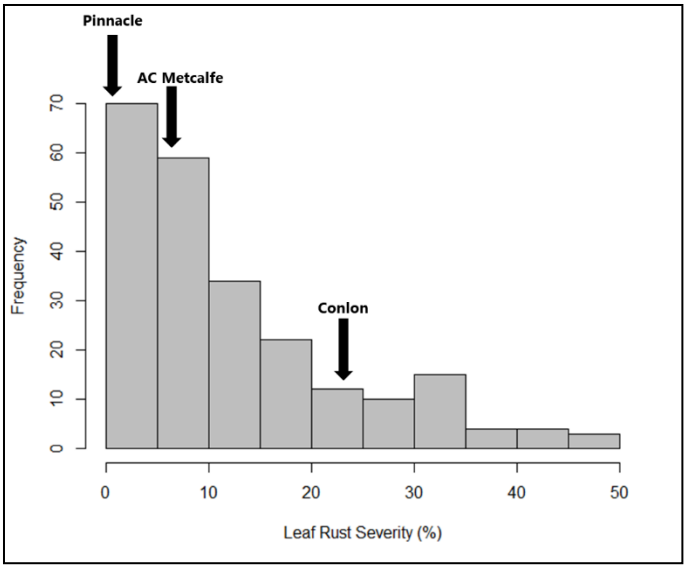

Figure 27. Histogram of the mean leaf rust severities of the 233 individuals in the MET population from the two replicates of the screen planted in the field in 2017. The black arrows represent the range of disease observed in the checks by showing the mean leaf rust severity of each of the check varieties with the highest, median, and lowest leaf rust severity. There were seven check varieties.

response in 2016 and 2017, but in 2017, Pinnacle had the lowest leaf rust at 0.4% and Conlon had the highest rust with 23.5% (Table 64). None of the MET individuals fell below this range, but several were more susceptible, with one reaching 50%, the maximum severity observed in 2017 (Fig. 27).

Table 63. Significance of main effects and the interaction term from the ANOVA run on the leaf rust scores of the checks in the 2017 field MET trial.

Effect	p-value	Level of significance[k]
Variety	1.80E-05	***
Location	5.21E-01	n.s.
Variety × Location	0.000233	***

[k] n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p<0.001$

Table 64. Mean leaf rust severities for the check cultivars, averaged across the two replicates of the MET planted in the field in 2017.

Variety		Mean leaf rust severity (%)
Conlon	a[x]	23.5
Hockett	ab	15.8
CDC Copeland	abc	14.2
Conrad	bcd	6.7
AC Metcalfe	bcd	6.0
LCS Genie	bcd	4.0
AAC Synergy	bcd	3.3
ND Genesis	cd	2.0
Pinnacle	d	0.4

[x] Varieties followed by the same letter do not statistically differ, $P = 0.05$, Tukey's HSD

The same two peaks were identified in both GWA analyses for leaf rust (Fig 27). One was located on the long arm of chromosome 2H, with 15 significant SNP markers in the 2016 analysis and 14 significant SNP markers in 2017. The other peak was located on the long arm of chromosome 5H, with five significant markers in 2016 and only one in 2017 (Fig. 27, Tables 65 and 66). The variance and the allelic effect of the markers were only calculated for the markers

with highest significance in each peak. They had phenotypic variances explained ranging from 30.0% to 52.8% and allelic effects ranging from 30.1% to 43.9% (Tables 65 and 66). The PCs run in each model adequately accounted for the population structure in the genetic data (Fig. 28).

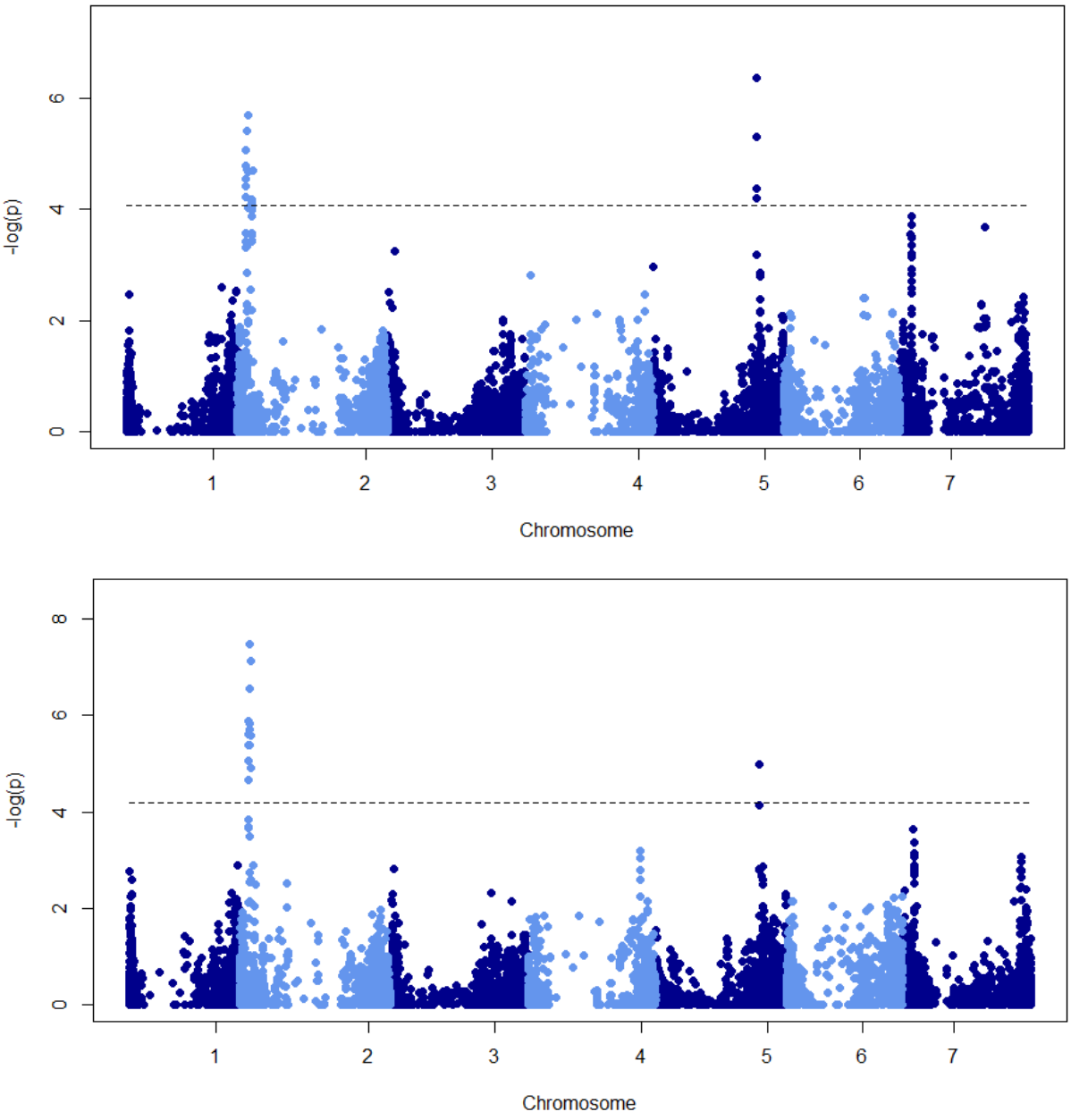

Figure 27. Manhattan plot of the –log(*p*-values) calculated for the 8,882 SNPs tested, mapped to their position in the barley genome. The p-values were obtained from the genome-wide association study performed on the leaf rust severity scores of 223 two-row spring barley lines infected with the disease from natural inoculum in the summer of 2016 (top) and in the summer of 2015 (bottom). Plants were rated for disease at the adult stage. The dashed line represents a false discovery rate cut-off of 5%. Only the points above the line are considered significant.

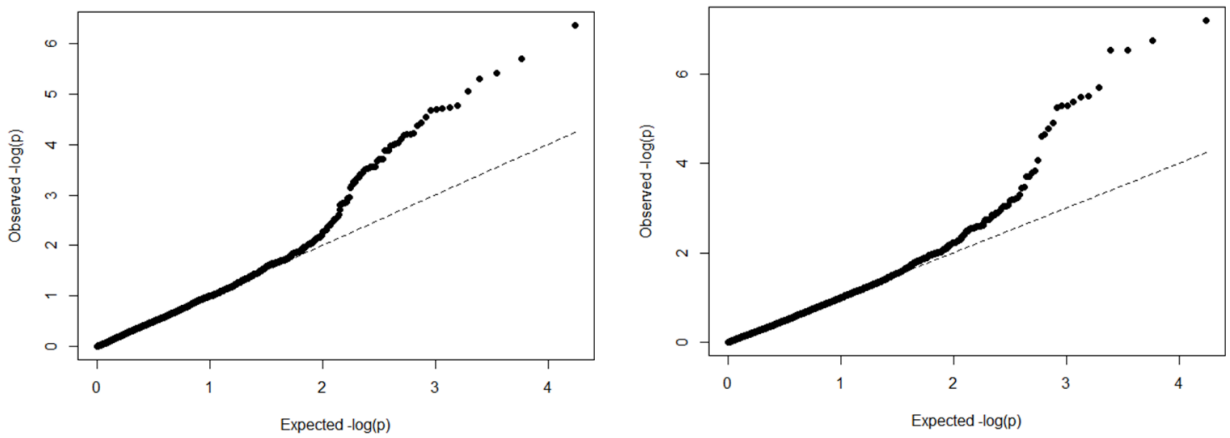

Figure 28. QQ plots of the –log(*p*-values) for 8,882 SNPs obtained from the genome-wide association analyses performed on the leaf rust disease severity of 223 members of the MET population in 2016 (left) and 2017 (right).

Table 65. Description of significant markers in the 2016 leaf rust GWA analysis.

Marker	Alleles[f]	Chromosome	Position	cM Position	-log(p)	p-value	R^2 (%)[n]	Allele Effect (%)[m]	Resistant Allele
S2_57048186	T/C	2H	57048186	61.55	5.69	2.02E-06	49.2	37.8	T
S2_48453125	G/A	2H	48453125	59.72	5.42	3.81E-06			
S2_46791106	G/T	2H	46791106	59.72	5.06	8.72E-06			
S2_45679335	C/G	2H	45679335	59.72	4.77	1.68E-05			
S2_52803590	A/G	2H	52803590	59.86	4.73	1.88E-05			
S2_52803720	A/G	2H	52803720	59.86	4.72	1.90E-05			
S2_79714857	C/A	2H	79714857	67.26	4.69	2.06E-05			
S2_54263832	G/A	2H	54263832	60.42	4.68	2.11E-05			
S2_47929263	C/T	2H	47929263	59.72	4.54	2.91E-05			
S2_44844192	C/A	2H	44844192	59.72	4.42	3.76E-05			
S2_45742754	T/C	2H	45742754	59.72	4.22	6.05E-05			
S2_75552464	G/A	2H	75552464	67.26	4.18	6.56E-05			
S2_78190074	T/C	2H	78190074	67.26	4.11	7.70E-05			
S2_77490310	T/A	2H	77490310	67.26	4.04	9.20E-05			
S2_57663422	G/T	2H	57663422	61.83	4.02	9.53E-05			
S5_527379438	T/C	5H	527379438	96.72	6.36	4.34E-07	30.0	42.0	C
S5_527381939	A/G	5H	527381939	96.72	5.31	4.94E-06			
S5_528046325	A/C	5H	528046325	97.21	4.19	6.41E-05			
S5_528046357	C/T	5H	528046357	97.21	4.19	6.41E-05			
S5_528362123	G/A	5H	528362123	97.44	4.38	4.18E-05			

[m] Allele effect = (the mean of infection responses of lines carrying susceptible alleles − the mean of infection responses of lines carrying resistant alleles) / the mean of infection responses of lines carrying susceptible alleles *100%

[n] The marker R^2 is the portion of phenotype variance explained by the marker, with no other terms included in the model. R^2 was only calculated for the most significant marker in each QTL

[p] Allele of the SNP marker found to be carried by the more-resistant individuals

Table 66. Description of significant markers in the 2017 leaf rust GWA analysis.

Marker	Alleles[f]	Chromosome	Position	cM Position	-log(p)	p-value	R^2 (%)[n]	Allele Effect (%)[m]	Resistant Allele[p]
S2_48453125	G/A	2H	48453125	59.72	7.48	3.29E-08	52.8	43.9	G
S2_57048186	T/C	2H	57048186	61.55	7.13	7.34E-08			
S2_50803532	A/G	2H	50803532	59.72	6.55	2.80E-07			
S2_50803573	C/G	2H	50803573	59.72	6.55	2.80E-07			
S2_47929263	C/T	2H	47929263	59.72	5.89	1.27E-06			
S2_52803720	A/G	2H	52803720	59.86	5.83	1.49E-06			
S2_52803590	A/G	2H	52803590	59.86	5.72	1.91E-06			
S2_45679335	C/G	2H	45679335	59.72	5.62	2.42E-06			
S2_54263832	G/A	2H	54263832	60.42	5.58	2.65E-06			
S2_51955296	A/G	2H	51955296	59.72	5.38	4.18E-06			
S2_45742754	T/C	2H	45742754	59.72	5.37	4.22E-06			
S2_44844192	C/A	2H	44844192	59.72	5.07	8.43E-06			
S2_57663422	G/T	2H	57663422	61.83	4.91	1.24E-05			
S2_46791106	G/T	2H	46791106	59.72	4.66	2.18E-05			
S5_527379438	T/C	5H	527379438	96.72	4.98	1.05E-05	16.2	30.1	C

[m] Allele effect = (the mean of infection responses of lines carrying susceptible alleles − the mean of infection responses of lines carrying resistant alleles) / the mean of infection responses of lines carrying susceptible alleles *100%

[n] The marker R^2 is the portion of phenotype variance explained by the marker, with no other terms included in the model. R^2 was only calculated for the most significant marker in each QTL

[p] Allele of the SNP marker found to be carried by the more-resistant individuals

The markers in LD with S2_48453125 (59.72 cM), the marker with peak significance for the leaf rust QTL on chromosome 2H in 2017 (Table 66), range from S2_44844192 (59.72 cM) to S2_57663422 (61.83 cM), representing a span of 12.8 Mbp or 2.11 cM (Fig. 29). The markers directly flanking this range of LD are S2_44543954 (59.72 cM) and S2_57663412 (61.84 cM), representing a span of 13.1 Mbp or 2.12 cM.

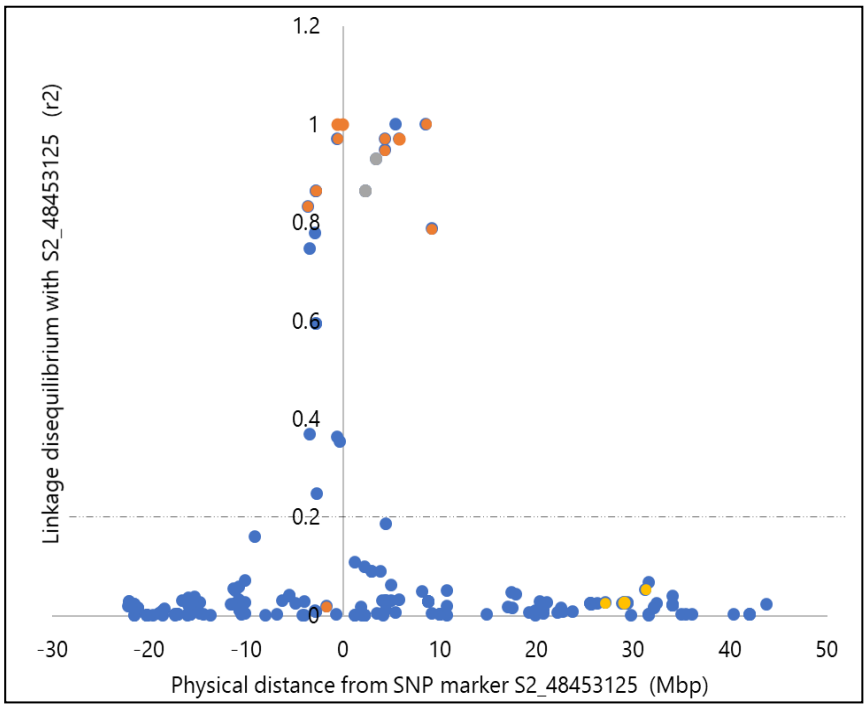

Figure 29. Plot of the linkage disequilibrium (LD) of the 100 markers located directly upstream and downstream of S2_48453125, the most significant marker found for adult resistance to leaf rust on chromosome 2H in 2017, and the second-most significant point in 2016. The dashed line is drawn at $r^2 = 0.2$, which was the cutoff for points in LD with the marker. The orange points are SNPs that were significant in both GWA analyses. The gray points were only significant in the 2017 analysis and the yellow points were only significant in the 2016 GWA analysis. The SNPs marking the boundaries of LD with S2_48453125 are S2_44844192 and S2_57663422, representing a span of 12.8 Mbp.

The markers in LD with S5_527379438 (96.72 cM), the marker with peak significance for the leaf rust QTL on chromosome 5H, range from S5_517378428 (92.45 cM) to S5_535404211 (101.59 cM), representing a span of 18.0 Mbp or 9.14 cM (Fig. 30). The markers directly flanking this area of the chromosome in LD with S5_527379438 are S5_517066952 (92.38 cM) and S5_535546782 (101.64 cM), representing a span of 18.5 Mbp or 9.26 cM.

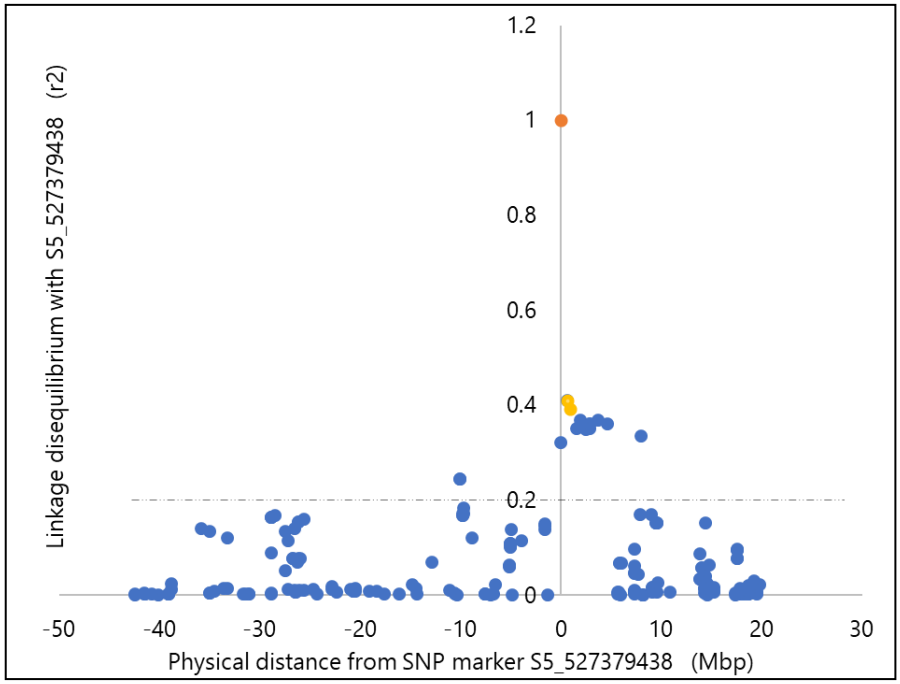

Figure 30. Plot of the linkage disequilibrium (LD) of the 200 markers located directly upstream and downstream of S5_527379438, the most significant marker for adult resistance to leaf rust on chromosome 5H. The dashed line is drawn at $r^2 = 0.2$, which was the cutoff for points in LD with the marker. The orange points are SNPs that were significant in both GWA analyses. The yellow points were only significant in the 2016 GWA analysis. The SNPs marking the boundaries of LD with S5_527379438 are S5_517378447 and S5_535404211, representing a span of 18.0 Mbp.

Discussion

Finding sources of resistance to spot blotch, powdery mildew, and leaf rust will be important as new barley lines are bred to be adapted to New York's climate. Using either a New York-collected isolate or natural inoculum in New York locations, five disease resistance QTLs were identified in the MET population, which was composed of elite germplasm from breeding programs in the Midwestern and Northwestern United States. Two of these QTLs, one on chromosome 2H and one on chromosome 7H, were found for seedling resistance to spot blotch using a single, aggressive isolate of *B. sorokiniana*. In field screens using natural inoculum, one QTL for adult resistance to powdery mildew was found on chromosome 1H, and the same two

QTLs for adult resistance to leaf rust were identified in 2016 and 2017. One of these QTLs was on chromosome 2H and the other was on 5H. The two QTLs on 2H for spot blotch resistance and leaf rust resistance are not colocalized.

The spot blotch QTL on 2H has previously been identified as playing a role in adult plant resistance in Bilgic et al. (2005), with the resistant allele coming from Harrington, a two row malting barley variety from Canada. This QTL, named *Rcs-qtl-2H-7-8*, explained 3% of the phenotypic variation. The same QTL was found again in Bilgic et al. (2006) with the donor allele coming from Calicuchima-sib, a line developed in Mexico. In this case, the allele explained 21% of the phenotypic variation. There are no previous reports of this QTL playing a role in seedling resistance. The spot blotch resistance QTL on 7H has been identified in several studies. The first was Steffenson et al. (1996) who named it the *Rcs5* locus. For seedling resistance, the resistant allele came from Morex, and the locus explained 71% of the phenotypic variation. For adult resistance, the same locus was found to explain 12% of the phenotypic variation. The *Rcs5* locus has also been found in four biparental populations screened as seedlings and as adults in Australia (Bovill et al. 2010), in a screen of wild barley (Roy et al. 2010), and in a screen of the USDA core collection with a pathotype 1 *B. sorokiniana* isolate (Wang et al. 2017). This locus is one of three loci that are collectively responsible for the durable resistance found in North American six-row varieties (Zhou and Steffenson 2013).

The QTL on 1H found for powdery mildew resistance is not in the location of any known major resistance gene for powdery mildew resistance. It may colocalize with a QTL, *QPm.YeFr-1H*, found in a biparental cross of two Australian cultivars, Yerong, a six-row feed variety, and Franklin, a two-row malting variety. This QTL accounted for 66% of phenotypic variation in an

adult plant screen of powdery mildew (Li and Zhou 2011), which is almost twice as much variation as was explained by the QTL found in this study.

Neither of the leaf rust resistance QTLs identified in this study colocalize with any of the 23 major leaf rust resistance genes in barley. Beyond the 23 major resistance loci, at least 50 QTLs have been identified for leaf rust resistance in seedlings, adult plants, or both (Qi et al. 1998; Kicherer et al. 2006; Jafary et al. 2006; von Korff et al. 2005; Marcel et al. 2007; Hickey et al. 2011; Castro et al. 2012; Gonzalez et al. 2012; Ziems et al. 2014, 2017; Gutiérrez et al. 2015; Singh et al. 2015, 2017). Of these, fewer than half been found in overlapping regions of the barley genome. This gives a strong indication that quantitative resistance to leaf rust is a complex trait with abundant and diverse QTLs underlying the resistance (Qi et al. 2000). The QTLs from this study identified on chromosome 2H may colocalize with *Rphq18*, a locus found in a doubled haploid population from Oregon and in a cross between a western European cultivar and an Ethiopian landrace (Marcel et al. 2007; González et al. 2012), but it lies on the edge of the confidence interval for this locus. The interval reported in González et al. (2012) spanned from 50 to 60 cM, while the QTL in this study was found to lie between 59.7 and 61.8 cM. There are no reports of loci colocalizing with the leaf rust resistance QTL identified on chromosome 5H, located between 92 and 102 cM, with a peak at 96 cM. The closest QTL previously identified is located at 89 cM in Latin American germplasm (Gutiérrez et al. 2015).

There are trade-offs between using greenhouse evaluations of disease resistance and screening for disease resistance in the field using only natural inoculum. One advantage of the field screen was that the MET was exposed to a disease population rather than a single isolate, as was done in the spot blotch greenhouse evaluation. To improve spot blotch evaluations, the pathotypes of. *B. sorokiniana* existing in New York should be determined to better target what

genetic resistance is needed. And, while it was useful that the plants in the field were exposed to populations of the pathogen, the field screens are subject to greater variation than greenhouse experiments. For example, two locations where the MET was planted in 2016 were not ratable because drought conditions did not allow for disease to develop. Also, field ratings for Fusarium head blight in 2016 and 2017 and spot blotch in 2017 were analyzed using GWA. For these analyses, variety was significant in the ANOVAs of the check varieties, but no significant SNPs were found in the GWA analyses. This indicates that non-inoculated field trials may work for disease resistance conferred by genes with large effects, but do not work as well for diseases with complex resistance.

The QTLs identified in this study could be incorporated into New York-adapted two-row spring malting barley using marker-assisted selection to provide durable resistance to spot blotch, powdery mildew, or leaf rust. Additionally, these loci could be used to investigate the mechanisms of quantitative resistance to these diseases. A model of how to study barley disease resistance mechanisms exists in leaf rust. One approach has been to examine the development of the leaf rust in plants with and without a given QTL (Wang et al. 2010). Also, some work has been done to fine-map leaf rust resistance QTL using near-isogenic lines (Yeo et al. 2017). Once a QTL's region is narrowed, putative genes within the region from BAC libraries (Yeo et al. 2016) or the annotated barley genome (Mascher et al. 2017) can be tested for their effect on disease response. Testing the function of individual genes within barley is becoming increasingly feasible using virus-induced gene silencing (Hein et al. 2005) or CRISPR/Cas9 technology (Lawrenson et al. 2015; Kapusi et al. 2017).

CHAPTER 5

EPILOGUE

The focus of this thesis was to determine which malting barley diseases are common in New York State, and to explore genetic resources for resistance to those diseases. Genetic resistance is only one tool that can be used to manage diseases. Other methods for control include chemical and cultural management, but the use of disease-resistant cultivars is particularly appealing because it can be more economically-viable and more environmentally-friendly than spraying fungicides and is typically more effective than using tilling or crop rotations to reduce primary inoculum.

The first product of this thesis was a reference, designed to be used by growers, outlining the disease susceptibilities of several winter and spring malting barley cultivars. In the winter barley, differences between cultivars were found for the scald, leaf rust, and powdery mildew, and Fusarium head blight (FHB). In spring barley, differences between cultivars were found for leaf rust, powdery mildew, spot blotch, and FHB. Scald was occasionally observed in spring barley and spot blotch was occasionally observed in winter barley, but these diseases tended to only cause significant damage to the upper leaves in winter barley for scald and in spring barley for spot blotch. And, while powdery mildew and leaf rust were present in winter barley, they tended to increase in severity in the spring barley, as long as the environmental conditions remained conducive to disease development.

The summary of cultivars' disease susceptibilities serves as an improvement over the previously-published annual Plant Disease Management Reports (PDMRs) because it was created using disease data from several locations over the 2015 – 2017 growing seasons. Disease

severity varied drastically from year-to-year and from location-to-location, which meant that any single year or trial was insufficient to draw conclusions about cultivars' susceptibilities. Also, for the most part, the cultivars included in the analysis were those that showed adequate agronomic characteristics to be kept in the trials for all three years, making the summary relevant to growers who must also consider factors such as yield and malting quality traits when selecting a cultivar to grow.

While analyzing the three years of disease data, spot blotch, caused by *Bipolaris sorokiniana*, was identified as a threat to spring barley. To prepare to do evaluations of cultivars for response to spot blotch, an aggressive isolate of *B. sorokiniana* collected in New York was identified, methods to produce inoculum were compared, and seedling and adult cultivars were evaluated for their response to two *B. sorokiniana* isolates. While evaluating the seedling and adult cultivars, a strong isolate × cultivar interaction was observed in the seedlings, and some of the adult cultivars exhibited a different level of susceptibility to spot blotch in the greenhouse than they did in the field where they were exposed to a population of *B. sorokiniana*. These two pieces of evidence indicate that *B. sorokiniana* has several patterns of pathogenicity, or pathotypes, in the state.

The third set of findings from this body of research were five quantitative trait loci (QTLs) identified for disease resistance to spot blotch, leaf rust, and powdery mildew in a diverse set of elite breeding lines of spring two-row malting barley compiled at the University of Minnesota. Two QTL were found when the population was evaluated as seedlings for spot blotch resistance using a single isolate of *B. sorokiniana*. The spot blotch resistance QTL identified on chromosome 7H colocalizes with one of the first QTL discovered for spot blotch resistance in barley, named *Rcs5* by Steffenson et al. (1996). This QTL has previously been described as

playing a role in both seedling and adult resistance. The second QTL identified for spot blotch resistance was on chromosome 2H, and has only previously been described as a QTL for adult plant resistance to spot blotch.

A single QTL on chromosome 1H was identified when the population was screened for powdery mildew resistance in the field. This QTL does not co-localize with any known major resistance genes for powdery mildew in barley, but it does co-localize with a QTL found in a bi-parental population created with a cross of a feed and a malting barley variety from Australia (Li and Zhou 2011). Finally, the same two QTLs were identified in 2016 and 2017 for leaf rust resistance when adult plants were screened for leaf rust in the field. One QTL was found on chromosome 2H and the other was found on chromosome 5H. Neither of these QTLs co-localize with any of the major resistance genes previously identified for either seedling or adult leaf rust resistance. Numerous loci involved in partial resistance to leaf rust have also been identified, but none clearly colocalize with those identified in this thesis.

Research stemming from this thesis could take several directions. One aspect that should be further explored is disease resistance to Fusarium head blight (FHB). In the scope of the research completed for this thesis, only visual ratings of FHB were considered to assess susceptibility. This is problematic because kernels infected with *Fusarium* do not always exhibit strong symptoms, symptoms can be confused with those of other diseases, and visual symptoms do not always give a good indication of the quantity of deoxynivalenol (DON) produced in the heads. The technologies to better characterize the degree of FHB infection exist, but are more expensive in terms of time and resources. For example, a different way to analyze incidence is to collect barley heads and allow the fungi growing internally to sporulate or grow onto media. This is a more definitive way of identifying the fungi causing symptoms or to identify pathogens that

are not causing obvious symptoms. Another way to test the level of infection is to analyze samples of ground barley using quantitative PCR with *Fusarium* species-specific primers or probes. Deoxynivalenol and other mycotoxins can be quantified using gas chromatography–mass spectrometry. Since these methods have a high cost compared to visual observation, the cultivars, locations, and years tested would have to be chosen carefully to provide the most useful information.

The focus on spot blotch in this thesis could mistakenly imply that spot blotch is the most damaging foliar disease of barley in the state. This is not the case, especially not in winter barley. Two winter barley cultivars, Flavia and KWS Scala, were determined to be highly susceptible to scald. Both varieties are available for sale in New York, and could be considered as potential parents for a breeding population if winter barley were to be bred in the state. Therefore, it could be worthwhile to prepare to do experiments with the scald pathogen, *Rhychosporium commune*. To do so, *R. commune* isolates should be collected from around the state and evaluated for pathogenicity in a controlled environment.

There is evidence that the isolates of *B. sorokiniana* used in this thesis to evaluate cultivars' spot blotch susceptibility have different pathotypes. Since only two isolates were used to evaluate multiple cultivars, there is a good chance that there are more pathotypes of *B. sorokiniana* in New York that have yet to be tested on the parents of the Cornell University's two-row breeding population. More isolates collected in New York should be screened using the twelve-cultivar panel from this thesis to ensure that the isolates used in future evaluations are representative of the local *B. sorokiniana* population.

Cornell University's two-row spring barley breeding population is being genotyped using genotyping by sequencing in the winter of 2017-18. This population was also screened for

response to leaf rust in the summer of 2017. Genome-wide association analysis should be run using the leaf rust phenotypes to determine whether either of the two disease-resistance QTLs identified in the Minnesota MET population are also in Cornell's breeding lines. If either is present in the breeding population, presence or absence of the QTL could be used as a selection criterion. Alternatively, if they are not present in the breeding material, there is potential for these QTLs to be incorporated into future New York-adapted cultivars to provide durable resistance to leaf rust. If conditions are conducive for disease development, spot blotch and powdery mildew could be screened in future years to do similar tests.

The precision of gene editing tools is improving quickly, so understanding the function of quantitative disease resistance loci could lead to new ideas about how to manipulate the barley genome to block disease development. Therefore, the mechanisms underlying the QTLs identified in this thesis should be investigated. A common method used thus far to characterize QTLs has been to develop near-isogenic lines (NILs) that only vary at the QTL and observe the development of the disease in the different NILs. Producing NILs requires several generations of backcrossing and selfing, so alternatively, a bulk analysis of lines with different genetic backgrounds some with or without the QTL could be observed to see if there are common differences in disease development in the two populations. Also, the annotated barley genome has many putative genes at the QTLs identified in this thesis, many of which are in families associated with disease resistance. It is becoming increasingly feasible to test whether these genes are involved in disease resistance using precise knock-out and gain-of-function experiments with highly-transformable lines of barley.

APPENDIX

PLANT DISEASE MANAGEMENT REPORTS

BARLEY (*Hordeum vulgare*)
Fusarium head blight; *Fusarium graminearum*
Leaf rust; *Puccinia hordei*
Spot blotch; *Bipolaris sorokiniana*
Scald; *Rhynchosporium secalis*

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Evaluation of Fusarium head blight and foliar diseases on winter malting barley varieties in New York, 2015.

Winter malting barley trials were conducted at Helfer and Ketola farms in Ithaca, NY. Each trial was conducted in a randomized complete block design with 2- and 6- row varieties interspersed. Three replicates were planted at Ketola and four replicates were planted at Helfer. Plots were 13 ft long and 6 rows wide with 7-in. row spacing. Seed was sown at a rate of 96 lb/A on 25 Sep at Helfer and 30 Sep at Ketola. Fields were prepared with a 300 lb/A pre-plant application of 10:20:20 (delivering 30 lb/A of nitrogen), and in the spring were top-dressed with 150 lb/A of 34:0:0 (delivering 51 lb/A of nitrogen). No fungicides or insecticides were applied over the course of the trials. Broadleaf herbicide (Maestro 2EC and Harmony Extra SG, with Induce) was applied in early April. No artificial inoculations were performed. Foliar disease severities were estimated across the plot as percentage of the top two leaves affected. Fusarium head blight (FHB) incidence was estimated by counting the number of symptomatic heads out of 50. Foliar diseases and FHB incidence were evaluated on 19 Jun at Ketola and 20 Jun at Helfer. Plants were at the hard dough stage of development at the time of rating. Disease incidence and severity means were analyzed with analysis of variance and separated by Tukey's HSD test (P=0.05).

The lowest incidence of FHB was consistently observed in the line 'AC 07/022/2', and the greatest incidence was observed in '03/220/158'. While 'AC 07/022/2' had the lowest FHB incidence, it also consistently had the greatest spot blotch severity. Scald was only observed to have significant results

in Ketola, where ‘KWS Scala’ had a greater severity rating than any other variety. Leaf rust was present at both locations, but there was no difference observed in its severity between varieties. Despite the high incidence of FHB, especially at Helfer, and the presence of spot blotch on every barley variety, no other clear patterns emerged from the disease ratings. This indicates that it may be difficult to determine the best varieties to provide resistance to diseases in New York.

		FHB incidence (%)				Spot blotch (%)				Scald (%)	
Entry	Rows	Helfer[x]		Ketola		Helfer		Ketola		Ketola	
02Ab431	2	41.50	abcde	8.67	c	11.25	ab	2.33	b	0.00	b
02Ab669	2	32.50	cde	8.00	c	15.00	ab	2.67	b	0.67	b
02Ab671	2	37.00	cde	8.00	c	6.25	b	2.67	b	0.00	b
2Ab08-X05W061-208	2	39.00	bcde	16.67	bc	12.50	ab	2.00	b	0.00	b
2Ab08-X05W061-216	2	45.50	abcde	22.66	bc	12.50	ab	5.00	b	0.00	b
AC 07/022/2	2	20.50	e	5.33	c	22.50	a	25.00	a	0.00	b
AC 07/041/33	2	43.50	abcde	19.33	bc	13.33	ab	6.50	ab	0.00	b
AC 07/041/8 (Flavia)	2	56.00	abcd	50.66	ab	7.50	b	8.50	ab	0.00	b
Charles	2	39.50	bcde	30.66	abc	10.75	ab	2.17	b	0.00	b
Endeavor	2	28.50	de	17.33	bc	10.75	ab	10.67	ab	0.17	b
KWS Scala	2	46.00	abcde	18.00	bc	5.00	b	3.33	b	4.67	a
KWS Stella	2	44.00	abcde	30.66	abc	3.00	b	8.00	ab	0.00	b
Nectaria	2	44.00	abcde	16.67	bc	16.67	ab	6.67	ab	0.00	b
SY Mezmaar	2	42.00	abcde	13.33	bc	15.00	ab	2.00	b	0.33	b
SY Tepee	2	53.00	abcd	27.34	bc	12.50	ab	4.00	b	0.00	b
WintMalt	2	46.00	abcde	25.34	bc	7.00	b	3.33	b	1.00	b
03/220/158	6	67.50	a	68.66	a	3.00	b	2.17	b	0.00	b
10467p2	6	60.66	abc	28.00	bc	13.33	ab	4.67	b	0.00	b
10467r2	6	47.00	abcd	27.34	bc	7.50	b	5.00	b	0.00	b
10467r4	6	63.50	ab	34.66	abc	12.00	ab	4.33	b	0.00	b
6Ab08-X03W012-5	6	35.34	cde	23.34	bc	6.50	b	2.33	b	0.00	b
Saturn	6	36.50	cde	41.34	abc	3.00	b	9.00	ab	0.00	b
HSD (*P*=0.05)		28.66		39.77		13.90		20.05		2.38	

[x] Column numbers followed by the same letter are not significantly different at *P*=0.05 as determined by Tukey’s HSD.

BARLEY (*Hordeum vulgare*)
Fusarium head blight; *Fusarium graminearum*
Leaf rust; *Puccinia hordei*
Powdery mildew; *Blumeria graminis* f. sp. *hordei*
Spot blotch; *Bipolaris sorokiniana*
Scald; *Rhynchosporium secalis*

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Evaluation of foliar diseases and Fusarium head blight on spring malting barley varieties in New York, 2015.

Spring malting barley variety trials were conducted at four locations: Ketola and Snyder farms in Ithaca, Tompkins County, NY; Batavia, Genesee County, NY; and Wayland, Steuben County, NY. These locations will be referred to as Ketola, Snyder, Genesee, and Steuben, respectively. Most of the barley varieties planted consisted of either 2- or 6-row malting barley, except for the 6-row feed varieties, 'Bastille' and 'Harmony'. Each trial was conducted in a randomized complete block design with three replicates. Plots were 13 ft long and 6 rows wide with 7-in. row spacing. The seeding rate was 96 lb/A for all trials. Trials were planted 28 Apr at Snyder, 29 Apr at Steuben, 5 May at Ketola, and 11 May at Genesee. Fields were prepared with a 300 lb/A pre-plant application of 10:20:20 (delivering 30 lb/A of nitrogen). No fungicides or insecticides were applied over the course of the trials. Broadleaf herbicide (Maestro 2EC and Harmony Extra SG, with Induce) was applied in early May. No artificial inoculations were performed. Foliar disease severities were estimated across the plot as percentage of symptoms on the top two leaves. Fusarium head blight (FHB) incidence was estimated by counting the number of symptomatic heads out of a representative 25. Foliar diseases were evaluated on 3 Jul at Snyder and 7 Jul at Ketola. FHB incidence was estimated on 8 Jul at Snyder and 15 Jul at Ketola. Foliar diseases and FHB were evaluated on 17 Jul at Genesee and Steuben. Disease incidence and severity means were analyzed with analysis of variance and separated by Tukey's HSD test (P=0.05).

Very little differentiation in FHB incidence was observed between varieties, suggesting that all varieties are susceptible under favorable conditions. The greatest differentiation between varieties was observed in powdery mildew severity. 'Lacey' was consistently observed to have greater levels of

powdery mildew. ‘M152’ and ‘Quest’ also had greater levels of powdery mildew than most other varieties at two of the locations. Notably, 6-row barleys had the greatest severity ratings for powdery mildew, while 2-row barleys were largely unaffected by the disease. No single variety had consistently greater spot blotch ratings at all locations, but ‘Scarlet’ had a greater disease severity than most other varieties at Snyder, and ‘KWS’ Tinka had a similar result at Genesee. For scald, ‘M152’ was observed to have a significantly greater severity than most other varieties at Snyder. In addition, leaf rust was present at low levels at every location, but there was no difference in severity observed between varieties. It may be difficult to identify spring malting barley varieties that will provide resistance to FHB and foliar diseases, other than powdery mildew, in New York environments.

		FHB incidence (%)						Powdery mildew (%)						Spot blotch (%)						Scald (%)			
Entry	Rows	Ketola[x]		Snyder		Genesee		Ketola		Snyder		Steuben		Snyder		Genesee		Steuben		Snyder		Genesee	
2ND28065	2	22	abc	0.67	b	22.66	abc	0.67	c	0.17	bc	18	bc	0.33	b	1	b	1.67	ab	0.83	b	0	b
AAC Synergy	2	1.33	c	0.33	b	18	abc	0.33	c	0	c	0.83	c	0.17	b	0.5	b	1	ab	0.33	b	0	b
Cerveza	2	2.67	bc	0.67	b	34	abc	0	c	0	c	0.83	c	0.33	b	1	b	0.83	b	0	b	0	b
Conlon	2	44	abc	9.33	ab	5.33	c	0	c	0	c	0.5	c	4.67	ab	1.67	ab	1	ab	0.67	b	0	b
Craft	2	27.34	abc	0.33	b	48.66	abc	0.5	c	0	c	2.33	c	0.17	b	3	ab	1.67	ab	2.33	ab	0	b
Herta	2	12	abc	3	ab	34.66	abc	0	c	0	c	0.17	c	1.5	ab	3.67	ab	1.67	ab	0.5	b	0	b
KWS 13/207	2	46	a	1.67	b	44.66	abc	0	c	0	c	0	c	0.83	b	1.67	ab	3.67	ab	0.33	b	0	b
KWS 13/3353	2	40.66	abc	13.33	ab	34.66	abc	0	c	0	c	0.5	c	6.67	ab	2.33	ab	3.67	ab	2.33	ab	0	b
KWS Amadora	2	19.33	abc	8	ab	45.34	abc	0	c	0	c	0.33	c	4	ab	0.83	b	1	ab	0.83	b	0	b
KWS Tinka	2	26.66	abc	1	b	52.66	ab	0	c	0	c	0	c	0.5	b	4.33	a	1.67	ab	0.83	b	0	b
ND Genesis	2	43.34	abc	0.33	b	56	ab	0	c	0	c	1	c	0.17	b	2.33	ab	1	ab	1.67	ab	0	b
Newdale	2	12	abc	0	b	19.33	abc	0.17	c	0	c	1.67	c	0	b	1	b	1	ab	0.33	b	0	b
Pinnacle	2	24.66	abc	4	ab	56.66	ab	0.17	c	0	c	3.67	c	2	ab	2.33	ab	9.33	a	0.83	b	0	b
Scarlett	2	12.67	abc	25	a	20	abc	0.17	c	0	c	0	c	12.5	a	1.67	ab	4	ab	0.33	b	0.67	a
Bastille	6	24.66	abc	0.67	b	44.66	abc	0	c	0	c	1.33	c	0.33	b	1	b	0.83	b	0.67	b	0	b
Harmony	6	44.66	ab	0	b	56.66	ab	0.17	c	0	c	1.67	c	0	b	0.83	b	0.83	b	1	b	0	b
Lacey	6	16	abc	0	b	48.66	abc	15	a	0.67	a	46.67	ab	0	b	1	b	2.33	ab	2.17	ab	0	b
M152	6	43.34	abc	0	b	21.34	abc	12.33	ab	0.17	bc	58.33	a	0	b	0.67	b	3.67	ab	5.33	a	0	b
M159	6	16	abc	0	b	15.33	bc	5	bc	0.5	ab	18.33	bc	0	b	0.5	b	2.33	ab	1	b	0	b
ND26891	6	42.66	abc	1	b	62.66	a	3	c	0.5	ab	12.67	c	0.5	b	0.67	b	4.67	ab	1.5	ab	0	b
Oceanik	6	21.34	abc	0.33	b	38.66	abc	0.17	c	0	c	2.17	c	0.17	b	0.67	b	4.33	ab	0.67	b	0.17	b
Quest	6	16	abc	1	b	22.66	abc	2.33	c	0.5	ab	63.33	a	0.5	b	1.5	ab	2.33	ab	0.83	b	0	b
Rasmusson	6	31.34	abc	0.67	b	32	abc	7.33	abc	0.5	ab	20	bc	0.33	b	0.83	b	4.33	ab	2.33	ab	0	b
HSD (P=0.05)		43.18		22.92		45.99		7.89		0.46		33.81		11.46		3.28		8.37		3.85		0.27	

[x] Column numbers followed by the same letter are not significantly different at P=0.05 as determined by Tukey's HSD.

BARLEY (*Hordeum vulgare*, multiple cultivars)
Leaf rust; *Puccinia hordei*
Powdery mildew; *Blumeria graminis* f. sp. *hordei*
Scald; *Rhynchosporium secalis*
Stagonospora leaf blotch; *Parastagonospora nodorum*

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Evaluation of foliar diseases on winter malting barley varieties in New York, 2016.

Winter malting barley trials were conducted at four central and western New York locations including Waterloo, Le Roy, and the Snyder and Ketola farms in Ithaca. Each trial was conducted in a randomized complete block design with 3 replicates and with 2- and 6- row varieties interspersed. Plots were 13 ft long and 6 rows wide with 7-in. row spacing. Seed was sown at a rate of 96 lb/A on 23 Sep at Snyder and 5 Oct at Ketola. Fields were prepared with a 200 lb/A pre-plant application of 10:20:20 fertilizer (delivering 20 lb/A of nitrogen). There was no topdress application of fertilizer in the spring. No fungicides or insecticides were applied over the course of the trials. Broadleaf herbicide (Maestro 2EC and Harmony Extra SG, with Induce) was applied in early April. No artificial inoculations were performed. Foliar disease severities were estimated across each plot as percentage of the top two leaves affected. Foliar diseases were evaluated on 1 Jun at both Snyder and Ketola, except for scald at Snyder, which was additionally rated on 24 May. Plants were at the hard dough stage of development at the time of rating. Disease severity means were analyzed with analysis of variance and separated by Fisher's LSD test (P=0.05). No results are reported for Waterloo or Le Roy locations due to insufficient levels of disease.

Scald was ultimately the disease with the greatest severity, reaching 21.7% severity in KWS Scala and Charles at Snyder on 1 Jun. However, results were not found to be statistically significant due to high variability of disease severity across the field except for at Snyder on 24 May (reported). KWS Scala and Charles were also the varieties with the greatest scald severity on 24 May. Powdery mildew was more severe at Snyder than Ketola. Nectaria stood out as the most susceptible variety overall, but at Snyder more 6-row than 2-row varieties had powdery mildew. Only two out of 11, 2-row varieties were observed to be infected by powdery mildew. In contrast, four of the seven 6-row barley lines had powdery

mildew. Stagonospora leaf blotch was only observed at Ketola and only one line, KWS2-430, had a significant amount of the disease (16.7% severity). Leaf rust was observed at both Ketola and Snyder, but severity was under 1% for all varieties at Ketola. At Snyder, leaf rust was most severe on the variety Charles and was found to be moderately severe on the MW11S3029-010 line. All other varieties had very little or no leaf rust.

		Leaf rust (%)	Powdery mildew (%)		Scald (%)	Stagonospora leaf blotch (%)
Entry	Rows	Snyder[x]	Snyder	Ketola	Snyder	Ketola
02Ab671	2	0.3 bc	1.3 bc	0.0 c	0.3 b	0.0 b
AC 07/041/8 (Flavia)	2	0.0 c	0.0 c	0.0 c	0.0 b	0.3 b
Charles	2	8.3 a	0.0 c	0.0 c	8.3 a	0.0 b
Endeavor	2	0.0 c	0.0 c	0.0 c	0.3 b	0.0 b
KWS Scala	2	0.0 c	0.0 c	0.0 c	8.3 a	0.0 b
KWS Somerset	2	0.0 c	0.0 c	0.0 c	0.0 b	2.3 b
KWS2-430	2	0.0 c	0.0 c	0.0 c	0.3 b	16.7 a
Nectaria	2	0.0 c	5.0 a	1.0 a	0.0 b	0.0 b
SY Mezmaar (209-72)	2	0.0 c	0.0 c	0.0 c	0.0 b	0.7 b
SY Tepee (209-66)	2	0.0 c	0.0 c	0.0 c	0.0 b	0.7 b
WintMalt	2	0.0 c	0.0 c	0.0 c	0.7 b	0.0 b
06-OR-9	6	0.0 c	3.0 ab	1.0 a	0.0 b	0.0 b
10467r2	6	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b
2011-F5-141-5	6	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b
6Ab08-X03W012-5	6	0.3 bc	4.3 a	0.7 b	0.0 b	0.0 b
MW11S3029-010	6	4.7 ab	4.7 a	0.0 c	0.0 b	2.0 b
MW11S3034-006	6	1.3 bc	3.7 a	0.0 c	0.0 b	0.0 b
Saturn	6	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b
p-value		0.049	<0.001	<0.001	<0.01	<0.001
LSD ($P = 0.05$)		4.5	2.2	0.2	4.2	2.7

[x]Column numbers followed by the same letter are not significantly different at P=0.05 as determined by Fisher's LSD.

BARLEY (*Hordeum vulgare*; multiple cultivars)
Leaf rust; *Puccinia hordei*
Powdery mildew; *Blumeria graminis* f. sp. *hordei*
Spot blotch; *Bipolaris sorokiniana*
Fusarium head blight; *Fusarium graminearum*

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Evaluation of foliar diseases on spring malting barley varieties in New York, 2016.

Spring malting barley variety trials were conducted at four locations in central and western New York including Batavia, Wayland, and Helfer and Ketola farms in Ithaca. Most of the varieties and breeding lines were 2- or 6-row malting barley. Feed varieties, Bastille, Oceanik, and Harmony; and food varieties, AAC Starbuck and AAC Azimuth were also included. Each trial was conducted in a randomized complete block design with three replicates. Plots were 13 ft long and 6 rows wide with 7-in. row spacing. Trials were planted at a seeding rate of 96 lb/A on 20 Apr at Ketola and 28 Apr at Helfer. Fields were prepared with a 300 lb/A pre-plant application of 10:20:20 fertilizer (delivering 30 lb/A of nitrogen). No fungicides or insecticides were applied. Broadleaf herbicide (Maestro 2EC and Harmony Extra SG, with Induce) was applied in early May. No artificial inoculations were performed. Foliar disease severities were estimated across the plot as percent coverage of the top two leaves. Foliar diseases were evaluated on 18 Jul at Helfer and 8 Jul at Ketola. Although the Batavia and Wayland locations were checked in July, there were insufficient levels of disease to rate. In addition to foliar diseases, Fusarium head blight (FHB) was monitored over the course of grain ripening. Disease severity means were analyzed with analysis of variance and separated by Fisher's LSD test (P=0.05).

Overall, leaf rust was the disease with the greatest severity at all locations. Two lines, 6-row variety AAC Azimuth and the breeding line 2MS14_3345_009, were the most susceptible, while most other varieties had little to no leaf rust. Out of all varieties and locations, only 6-row line M160 at Ketola had a powdery mildew severity greater than 5%. Powdery mildew, spot blotch, and FHB were present at many locations, but at levels too low for significant ratings.

		Leaf Rust (%)				Powdery Mildew (%)	
Entry	Rows	Helfer[x]		Ketola		Ketola	
06N2-79	2	0.0	c	0.0	e	0.0	d
09N2-16	2	0.0	c	0.0	e	0.0	d
09N2-31	2	0.0	c	0.3	e	0.0	d
09N2-51	2	1.0	bc	0.0	e	0.0	d
09N2-58	2	3.3	bc	2.0	de	0.0	d
09N2-65	2	0.0	c	0.0	e	0.0	d
09N2-68	2	0.0	c	0.0	e	0.0	d
09N2-84	2	1.7	bc	6.7	abc	0.0	d
09N2-96	2	0.0	c	0.3	e	0.0	d
2MS14_3305-002	2	0.0	c	1.7	de	1.0	bcd
2MS14_3317-015	2	0.0	c	0.3	e	0.0	d
2MS14_3317-018	2	0.0	c	0.0	e	0.0	d
2MS14_3335-007	2	0.0	c	1.7	de	1.0	bcd
2MS14_3336-002	2	1.3	bc	1.7	de	0.0	d
2MS14_3336-018	2	0.3	c	3.3	b-e	1.0	bcd
2MS14_3342-018	2	1.7	bc	1.0	e	0.0	d
2MS14_3342-019	2	1.3	bc	2.7	cde	0.0	d
2MS14_3342-026	2	0.3	c	0.3	e	0.0	d
2MS14_3345-009	2	13.3	a	7.7	a	0.0	d
2ND28065	2	3.3	bc	0.7	e	0.0	d
AAC Starbuck	2	3.3	bc	1.7	de	0.0	d
AAC Synergy	2	0.0	c	0.0	e	0.0	d
Cerveza	2	0.0	c	0.0	e	0.0	d
Conlon	2	1.7	bc	3.3	b-e	0.0	d
Craft	2	1.0	bc	3.3	b-e	0.0	d
KWS 13/207 (Fantex)	2	0.0	c	0.0	e	0.0	d
KWS 13/3353(Beckie)	2	0.3	c	0.0	e	0.0	d
KWS Tinka	2	0.3	c	1.0	e	0.0	d
ND Genesis	2	0.0	c	0.0	e	0.0	d
Newdale	2	0.0	c	0.0	e	0.0	d
Pinnacle	2	1.0	bc	0.3	e	0.0	d
AAC Azimuth	6	17.0	a	7.0	ab	1.3	bc
Bastille	6	6.7	b	3.7	a-e	0.0	d
Harmony	6	3.3	bc	1.0	e	0.0	d
HS5617-11	6	0.0	c	0.3	e	0.0	d
Lacey	6	1.7	bc	0.0	e	1.7	b
M160	6	0.3	c	1.7	de	5.0	a
ND26891	6	0.3	c	0.0	e	0.3	cd
Oceanik	6	0.0	c	1.0	e	0.0	d
Quest	6	0.3	c	5.3	a-d	1.0	bcd
p-value		<0.001		<0.01		<0.001	
LSD (*P*=0.05)		6.0		4.2		1.2	

[x]Column numbers followed by the same letter are not significantly different at *P*=0.05 as determined by Fisher's LSD.

BARLEY (*Hordeum vulgare*)
Fusarium head blight; *Fusarium graminearum*
Leaf rust; *Puccinia hordei*
Powdery mildew; *Blumeria graminis* f. sp. *hordei*
Scald; *Rhynchosporium commune*

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Evaluation of Fusarium head blight and foliar diseases on winter malting barley varieties in New York, 2017.

Winter malting barley variety trials were conducted at four locations in Central and Western New York, including one trial each in Monroe and Seneca Counties and two trials in Tompkins County: one at the Ketola and the other at the McGowan Research Farm. Each trial was conducted in a randomized complete block design with 3 replicates. The 24 varieties were completely randomized within each replicate. Plots were 13 ft long and 6 rows wide with 7-in. row spacing. Seed was planted the previous autumn at a rate of 96 lb/A on 27 Sep in Monroe County, 28 Sep at McGowan, 6 Oct at Ketola, and 7 Oct in Seneca County. Before planting, 200 lb/A of 20:20:20 fertilizer was applied (delivering 40 lb/A of nitrogen). In the spring, the field was topdressed with 50 lb/A of nitrogen. A broadleaf herbicide (Harmony Extra and Bromoxynil with Induce) was applied in early April. No fungicides or insecticides were applied over the course of the trial and no artificial inoculations were performed. Foliar disease ratings were estimated as the percent of the top two leaves affected by the disease over the entire plot. Fusarium head blight (FHB) was scored by measuring the incidence and severity of the disease in each plot and calculating the FHB index. Incidence was estimated by looking at 25 heads, counting how many were symptomatic, and multiplying by 4. Severity for each plot was determined by estimating the average percentage of kernels with the disease on the symptomatic heads, reported as a whole number. FHB index is equal to incidence x severity/100. Foliar diseases were rated on 2 Jun at the Tompkins County locations, 5 Jun in Seneca County, and 18 Jun in Monroe Co. For these ratings, the barley was at the soft dough growth stage in Tompkins and Seneca County, and at the hard dough growth stage in Monroe County. FHB was rated on 18 Jun in Monroe and Seneca Counties where the barley was at the hard dough growth stage, and on 20 Jun in Tompkins County where the barley was at the ripening growth stage. Disease severities and FHB index were square root-transformed and the means of the transformed

data were analyzed with analysis of variance and separated by Tukey's HSD test (P=0.05). Scald and FHB were observed in Seneca County, but the levels were too low to report.

FHB index was consistently highest for 10467r2, a 6-row breeding line. KWS Scala had an equivalently high FHB index of 2.3 in McGowan, the location with the highest levels of FHB, but had very low levels of the disease in the other two trials, indicating genotype by environment effects. 'DH140082', 'SU Mateo', and 'MW11S3034-006' had consistently low levels of FHB, with index scores at or below 0.3 at all locations. Overall, scald was the foliar disease with the highest severity in 2017, reaching a peak of 75% severity in Monroe, and 60% severity in Ketola on KWS Scala. In McGowan, the variety with the highest scald severity was Charles, with a severity of 41.7%. KWS Scala and Flavia consistently were in the top 3 varieties with the most scald, and Charles, OSU10.0925, 10/069/1 were all consistently in the top 5. All other varieties had average scald severities under 10% at all locations. Powdery mildew was highest on DH1400882 and MW11S3034-006, a 2-row and 6-row breeding line, respectively. Nectaria, 6W11-0064, and 6Ab08-X03W012-5 showed relatively high levels of the disease, and several 6-row lines showed moderate levels of the disease, including 6W13-7041, 6W13-7041, Alba, and MW11S3029-010. Leaf rust only had reportable severity at Monroe, where MW11S3029-010 (53.3% severity), 6Ab08-X03W012-5 (43.3%), and Charles (23.7%) were the only lines with severities higher than 10%.

		FHB Index						Leaf Rust (%)		Powdery Midew (%)				Scald (%)					
Variety	Rows	Ketola[x]		McGowan		Monroe		Monroe		Ketola		McGowan		Ketola		McGowan		Monroe	
Charles	2	0.2	b	1.2	a-d	0.3	ab	23.7	ab	0.0	c	0.0	e	30.0	abc	41.7	a	3.3	c
DH130718	2	0.2	b	2.4	ab	0.3	ab	3.7	bc	1.0	c	8.3	bcd	0.0	d	0.0	b	0.2	c
DH130910	2	0.2	b	0.5	a-d	0.3	ab	2.0	bc	0.0	c	0.0	e	6.8	bcd	1.0	b	0.2	c
DH1400882	2	0.2	ab	0.1	d	0.0	b	0.7	bc	10.3	abc	53.3	a	8.3	bcd	0.0	b	0.2	c
Endeavor	2	0.1	b	0.6	a-d	0.1	ab	0.3	bc	0.0	c	0.8	cde	3.0	bcd	2.2	b	3.7	c
Flavia	2	0.6	ab	0.8	a-d	0.2	ab	0.3	c	0.0	c	0.5	cde	46.7	a	10.0	b	70.0	a
KWS Scala	2	0.1	b	2.3	a	0.1	ab	0.0	c	0.0	c	0.2	de	60.0	a	11.7	ab	75.0	a
KWS Somerset	2	0.1	b	0.7	a-d	0.4	ab	0.5	bc	0.0	c	0.7	cde	6.0	bcd	0.0	b	1.8	c
KWS2-430	2	0.3	ab	0.8	a-d	0.3	ab	0.2	c	0.0	c	0.8	cde	5.2	bcd	0.0	b	10.2	bc
Nectaria	2	0.4	ab	0.2	cd	0.3	ab	0.5	bc	5.0	abc	35.0	a	2.0	cd	0.0	b	0.2	c
OSU10.0925	2	0.6	ab	1.6	abc	0.3	ab	0.2	c	0.0	c	0.3	de	23.3	abc	3.3	b	65.0	a
SU-Mateo	2	0.1	b	0.2	cd	0.1	ab	0.7	bc	0.0	c	0.0	e	0.5	d	0.2	b	0.2	c
SY Tepee (209-66)	2	0.3	ab	0.8	a-d	0.1	ab	0.2	c	0.0	c	0.0	e	2.2	bcd	0.2	b	5.2	c
Vincenta	2	0.0	b	0.3	bcd	0.1	ab	0.0	c	0.0	c	0.3	de	3.5	bcd	1.2	b	8.7	c
06-OR-9	6	0.1	b	1.6	abc	0.0	b	0.3	c	2.0	abc	11.0	bc	0.2	d	0.0	b	0.3	c
10/069/1	6	0.4	ab	1.8	abc	0.1	ab	0.0	c	0.0	c	0.5	cde	26.7	ab	6.7	b	36.7	ab
10467r2	6	1.5	a	2.3	a	1.1	a	0.5	bc	0.0	c	0.0	e	6.8	bcd	0.3	b	0.3	c
6Ab08-X03W012-5	6	0.4	ab	0.6	a-d	0.1	ab	43.3	a	11.7	ab	26.7	ab	0.2	d	0.0	b	0.2	c
6W11-0064	6	0.3	ab	1.6	abc	0.3	ab	2.2	bc	6.7	abc	40.0	a	0.3	d	0.0	b	0.3	c
6W13-7041	6	0.1	b	0.5	a-d	0.0	ab	2.2	bc	0.8	bc	21.7	ab	0.3	d	0.0	b	0.2	c
Alba	6	0.1	b	0.9	a-d	0.0	b	2.2	bc	6.0	abc	26.7	ab	0.0	d	0.0	b	0.0	c
MW11S3029-010	6	0.2	ab	0.4	a-d	0.4	ab	53.3	a	3.8	abc	26.7	ab	7.7	bcd	0.2	b	0.0	c
MW11S3034-006	6	0.3	ab	0.0	d	0.1	ab	3.8	bc	15.0	a	41.7	a	0.3	d	0.2	b	0.5	c
Saturn	6	0.4	ab	0.7	a-d	0.2	ab	8.8	bc	0.0	c	0.2	de	0.0	d	0.2	b	0.2	c
p-value		<0.01		<0.001		0.034		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

[x]Columns followed by the same letter are not significantly different at *P*=0.05 as determined by Tukey's HSD

BARLEY (*Hordeum vulgare*)
Spot blotch; *Bipolaris sorokiniana*
Leaf rust; *Puccinia hordei*
Powdery mildew; *Blumeria graminis* f. sp. *hordei*

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Evaluation of foliar diseases on spring barley varieties in New York, 2017.

Spring malting barley variety trials were conducted at four locations in Central and Western New York. One trial was planted in Genesee County, another was planted in Steuben County, and the other two were planted in Tompkins County, one at the Helfer and the other at the Ketola Research Farm. Each trial was conducted in a complete randomized block design with 3 replicates. All 36 varieties and breeding lines were completely randomized within each replicate. The fields were prepared with an application of 120 lb/A 27:18:9 fertilizer (delivering 32 lb/A of nitrogen). Plots were 13 ft long and 6 rows wide with 7-in. row spacing. Seed was planted at 96 lb/A on 24 Apr at Ketola, 27 Apr in Steuben County, 28 Apr at Helfer, and 11 May in Genesee County. No fungicides were applied and no artificial inoculations were performed. The severities of the foliar diseases were estimated as the percent of the top two leaves showing symptoms or signs of the disease across the entire plot. Diseases were rated on 14 Jul at Helfer, on 15 Jul at Ketola, and on 16 Jul in Genesee and Steuben Counties. The barley was at the hard dough growth stage for all disease ratings. All disease severities were square root-transformed except for the ratings of leaf rust at Ketola. The means of the transformed or original data were analyzed with analysis of variance and separated by Tukey's HSD test (P=0.05).

There were reportable levels of spot blotch at all 4 locations. Odyssey, a 6-row malting variety, had the highest severity at Helfer and Steuben County, hitting a peak of complete leaf death in Helfer, but had below 5% spot blotch severity at Ketola and Genesee County. At Ketola, KWS 15/3716 had the highest spot blotch with 52.5% severity and in Genesee County, 07AB-59 had the highest severity at 11.7%. Both of these lines had mid- to high-severity at the other locations, as well. Helfer was particularly hard-hit by spot blotch, so that most varieties had higher than 10% severity. A few varieties, especially 6-rows, had no or very low levels of spot blotch at that location. The 6-row varieties, Quest

(malting barley), Bastile (feed barley), and AAC Azimuth (food barley) had no spot blotch at any location, and the 2-row variety AAC Synergy (malting barley) had only 3.7% severity spot blotch at Helfer and none at the other trial locations. Leaf rust was consistently highest on the 6-row feed barley varieties from Synagri, Bastile and Harmony. Most varieties had moderate severities of leaf rust (20-40%) at Ketola. The 2-row varieties, KWS 15/2650 and KWS 15/3716, consistently had the lowest leaf rust severities. Powdery mildew also had reportable severities at all 4 locations. Overall, Quest and M160 had the highest powdery mildew severities, both reaching over 80% in Steuben County. Craft was the only 2-row variety with powdery mildew severity over 50%, reaching 55% in Ketola.

		Spot Blotch (%)								Leaf Rust (%)								Powdery Mildew (%)							
Variety	Rows	Helfer[x]		Ketola		Genesee		Steuben		Helfer		Ketola		Steuben				Helfer		Ketola		Genesee		Steuben	
07AB-59	2	71.7	a-e	0.0	b	11.7	a	18.3	ab	0.7	bc	25.0	ab	0.7	abc			0.0	f	0.0	c	0.0	b	0.0	e
08MT-03	2	35.0	a-g	0.0	b	0.0	b	2.0	bcd	0.7	bc	35.0	ab	0.2	bc			2.0	ef	30.0	abc	0.0	b	23.7	b-e
08WA-32	2	46.7	a-g	0.0	b	0.0	b	1.7	bcd	1.7	bc	10.0	b	0.0	c			0.0	f	2.5	bc	6.7	ab	1.7	de
09N2-21	2	21.7	c-h	0.0	b	1.7	b	3.3	bcd	1.8	bc	25.0	ab	0.2	bc			0.3	ef	7.5	abc	3.3	ab	5.0	de
09N2-31	2	21.7	c-h	0.0	b	0.0	b	3.3	bcd	0.3	bc	15.0	b	0.3	abc			8.3	b-f	7.5	abc	0.2	ab	25.0	a-e
09N2-58	2	8.3	fgh	0.0	b	0.3	b	0.0	d	0.2	bc	40.0	ab	0.3	abc			3.3	ef	17.5	abc	0.3	ab	10.0	cde
09N2-65	2	50.0	a-f	0.0	b	0.2	b	3.3	bcd	0.2	bc	22.5	ab	0.2	bc			3.3	ef	25.0	abc	0.3	ab	23.3	a-e
09N2-66	2	3.7	fgh	0.0	b	0.0	b	0.3	cd	1.0	bc	20.0	ab	1.0	abc			0.3	ef	15.0	abc	0.3	ab	0.5	de
09N2-67	2	5.0	fgh	0.0	b	0.0	b	0.3	cd	0.5	bc	27.5	ab	0.8	abc			8.8	c-f	12.5	abc	0.5	ab	1.8	de
09N2-68	2	11.7	e-h	0.0	b	0.0	b	0.0	d	3.3	bc	15.0	b	0.8	abc			2.0	ef	35.0	abc	0.0	b	8.5	cde
09N2-96	2	16.7	d-h	0.0	b	0.0	b	0.2	d	0.2	bc	12.5	b	0.3	abc			3.3	ef	7.5	abc	0.0	b	3.5	de
2MS14_3305-002	2	6.7	gh	0.0	b	0.0	b	0.2	d	5.3	bc	20.0	ab	0.8	abc			13.3	b-e	45.0	abc	1.7	ab	30.0	a-e
2MS14_3317-015	2	33.3	a-g	0.0	b	0.2	b	0.5	cd	0.2	bc	30.0	ab	2.0	abc			0.0	f	5.0	bc	0.0	b	0.0	e
2MS14_3336-002	2	28.3	a-h	0.0	b	0.0	b	3.5	bcd	2.3	bc	40.0	ab	1.5	abc			0.0	f	0.0	c	0.0	b	0.0	e
2MS14_3336-018	2	10.0	e-h	0.0	b	0.0	b	0.2	d	3.7	bc	40.0	ab	0.8	abc			8.3	c-f	15.0	abc	0.2	ab	23.3	a-e
2MS14_3342-026	2	43.3	a-h	0.0	b	0.0	b	3.7	bcd	6.7	bc	20.0	ab	2.0	abc			6.7	def	12.5	abc	0.0	b	11.7	cde
AAC Synergy	2	3.7	fgh	0.0	b	0.0	b	0.0	d	0.5	bc	35.0	ab	0.0	c			0.5	ef	12.5	abc	0.0	b	6.8	de
CDC Clear	2	12.0	e-h	2.5	b	0.0	b	0.5	cd	1.7	bc	35.0	ab	3.5	ab			0.0	f	0.0	c	0.0	b	0.0	e
Cerveza	2	15.0	d-h	0.0	b	0.0	b	0.3	d	0.2	bc	20.0	ab	0.0	c			0.0	f	10.0	abc	0.0	b	6.8	de
Conlon	2	23.3	b-h	0.0	b	0.2	b	1.7	bcd	0.3	bc	45.0	ab	2.3	abc			0.0	f	0.0	c	0.0	b	1.7	de
Craft	2	5.0	fgh	0.0	b	0.0	b	0.0	d	6.7	bc	17.5	b	1.2	abc			26.7	a-d	55.0	ab	1.7	ab	46.7	a-d
KWS 15/2650	2	98.3	ab	5.0	b	0.2	b	5.3	bcd	0.0	c	2.0	b	0.2	bc			0.0	f	0.0	c	0.0	b	0.2	de
KWS 15/3716	2	93.3	abc	52.5	a	1.7	b	11.7	a-d	0.0	c	3.0	b	0.2	bc			0.0	f	0.0	c	0.0	b	0.0	e
KWS Tinka	2	80.0	a-d	0.5	b	0.0	b	3.7	bcd	0.0	c	12.5	b	0.2	bc			0.0	f	2.5	bc	1.7	ab	0.0	e
ND Genesis	2	33.3	a-g	2.5	b	0.2	b	3.7	bcd	0.5	bc	7.5	b	0.2	bc			1.7	ef	15.0	abc	0.0	b	0.0	e
Newdale	2	18.3	d-h	0.0	b	0.2	b	0.0	d	0.3	bc	25.0	ab	0.0	c			0.0	f	10.0	abc	0.0	b	2.2	de
Odyssey	2	100.0	a	2.5	b	1.7	b	33.3	a	0.0	c	10.0	b	0.0	c			0.0	f	0.0	c	0.0	b	0.0	e
Pinnacle	2	60.0	a-f	0.0	b	0.0	b	26.8	abc	1.8	bc	15.0	b	0.0	c			5.0	ef	7.5	abc	0.0	b	11.7	b-e
AAC Azimuth	6	0.0	h	0.0	b	0.0	b	0.0	d	7.3	bc	12.5	b	1.7	abc			33.3	ab	40.0	abc	5.0	ab	45.0	a-e
Bastile	6	0.0	h	0.0	b	0.0	b	0.5	cd	30.0	a	80.0	a	2.3	abc			0.0	f	0.0	c	0.0	b	1.7	de
Harmony	6	2.3	gh	0.0	b	0.0	b	0.0	d	8.3	ab	60.0	ab	3.7	a			0.0	f	2.5	bc	0.0	b	1.8	de
HS5617-11	6	2.3	gh	0.0	b	0.0	b	0.2	d	1.0	bc	40.0	ab	0.8	abc			1.8	ef	7.5	abc	0.0	b	0.5	de
M160	6	0.0	h	0.0	b	0.0	b	0.0	d	1.7	bc	5.0	b	0.2	bc			56.7	a	80.0	a	31.7	a	88.3	a
ND26891	6	0.0	h	0.0	b	0.0	b	0.0	d	0.5	bc	5.0	b	1.8	abc			31.7	abc	45.0	ab	10.0	ab	73.3	abc
Oceanik	6	2.3	gh	0.0	b	0.0	b	0.7	cd	1.0	bc	25.0	ab	1.5	abc			0.5	ef	5.0	bc	25.3	ab	16.8	cde
Quest	6	0.0	h	0.0	b	0.0	b	0.0	d	1.0	bc	30.0	ab	0.2	bc			68.3	a	40.0	abc	16.7	ab	85.0	ab
p-value		<0.001		<0.001		<0.001		<0.001		<0.001		<.01		<0.001				<0.001		<0.001		<0.01		<0.001	

[x]Columns followed by the same letter are not significantly different at *P*=0.05 as determined by Tukey's HSD

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